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Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: National Sciences Engineering and Research Council
Canadian Institutes of Health Research
Canada Research Chair in Glial Neuroimmunology

Title: Interleukin-1 cytokines are regulators of developmental microglial proliferation

Authors: *B. HAMMOND[1], R. MANEK[1], T. LANGE[1], C. THOMAS[1], K. LEE[1], A. CASTELLANOS-MOLINA[4], F. BRETHEAU[4], B. J. KERR[2], S. LACROIX[5], J. R. PLEMEL[3]; [2]Anesthesiol., [3]Dept. of Medicine, Div. of Neurol., [1]Univ. of Alberta, Edmonton, AB, Canada; [4]Univ. Laval, Quebec City, QC, Canada; [5]Mol. Med., Univ. Laval, Quebec, QC, Canada

Abstract: Microglia proliferate robustly during development to reach a sufficient density to execute their functions. Despite an appreciation for the importance of microglial proliferation in development, the factors that drive this proliferation remain largely unclear. Interleukin-1 (IL-1) cytokines—inflammatory cytokines often viewed as hallmarks of immune responses—are enriched in the developing brain and regulate microglial proliferation in non-developmental contexts. It may be that these hallmark inflammatory cytokines have undescribed functions in regulating the developmental proliferation of microglia. In our work, we find reduced microglial densities in the brain and spinal cord of early postnatal IL-1α and IL-1β knockout mice, though spinal cord microglial densities normalize to control levels by postnatal day 30 (P30). However, neither IL-1α nor IL-1β promote microglial proliferation in culture. We therefore hypothesize that IL-1α and/or IL-1β guide developmental microglial proliferation by promoting the release of proliferative factors, also known as mitogens, from astrocytes, a cell lineage that alters secretion in response to IL-1 treatment. In culture, astrocytes treated with either IL-1α or IL-1β release unknown factors that promote microglial proliferation. We are working to identify the mitogen(s) present in astrocyte conditioned media and will identify how the mitogen(s) modulate developmental microglial proliferation in vivo. Together, this work will illuminate the factor(s) that drives microglia to initially establish their homeostatic density to enable proper functioning throughout life.

Disclosures: B. Hammond: None. **R. Manek:** None. **T. Lange:** None. **C. Thomas:** None. **K. Lee:** None. **A. Castellanos-Molina:** None. **F. Bretheau:** None. **B.J. Kerr:** None. **S. Lacroix:** None. **J.R. Plemel:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.02

Topic: A.01. Neurogenesis and Gliogenesis

Title: The use of cortical organoids to characterize hESC-derived glial cells

Authors: J. VAN SICLEN, S. KRIKS, H. NETHERCOTT, W. L. AU, Y. MAURY, C. R. NICHOLAS, *A. BULFONE;
Neurona Therapeut., South San Francisco, CA

Abstract: Brain organoids are pluripotent stem cell-derived aggregates that model aspects of the developing human forebrain. Generating organoids also offers the opportunity to study human oligodendroglial development in vitro and gain insights into their remyelinating potential. Our team developed a high-throughput in vitro system using cortical organoids to test the migration, maturation, and myelination potential of human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells (OPCs) of a medial ganglionic eminence (MGE)-type lineage generated with multiple differentiation protocols. We optimized the organoid system by modulating WNT and SHH signaling to improve the efficiency of generating dorsal forebrain-type progenitors. These progenitors are then cultured to further mature the organoids into layered structures that exhibit consistently high expression of cortical markers PAX6, FOXG1, CITIP2, BRN2, TBR1, and TBR2. Off-target ventral forebrain markers were not significantly expressed. We then optimized the timing of OPC implantation into the cortical organoids and the culture conditions post-implant in vitro. Independent batches of OPCs, labeled with GFP, were analyzed at multiple timepoints post-implantation. OPC persistence, distribution, and maturation were assessed by immunocytochemistry for expression of on-target OPC (SOX10, PDGFRa) and oligodendrocyte (BCAS, MBP) markers, as well as off-target astrocyte (GFAP) and neuronal (TUJ1) markers. Migration was assessed by analyzing the cell density at implantation sites and the radial distribution of GFP+ OPCs. The cortical organoid system allowed the triage of different hESC-OPC derivation protocols and represents a promising high throughput screening platform.

Disclosures: **J. Van Siclen:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **S. Kriks:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **H. Nethercott:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **W.L. Au:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **Y. Maury:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **C.R. Nicholas:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **A. Bulfone:** A. Employment/Salary (full or part-time):; Neurona Therapeutics.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NIDCD Grant DC007695

Title: Maturation of astrocytes in the medial nucleus of the trapezoid body

Authors: *E. M. AMICK[1], D. HELLER[1], P. S. HOLCOMB[3], V. MOSES[1], A. GABBARD[1], M. ATTALLA[1], M. H. ELLISMAN[4], G. A. SPIROU[2];
[2]Med. Engin., [1]Univ. of South Florida, Tampa, FL; [3]Sensory Neurosci. Res. Ctr., Sch. of Med., Morgantown, WV; [4]Dept Neurosci, Sch. of Med., La Jolla, CA

Abstract: Mature astrocytes have several defining features, including endfoot processes that contact blood vessels as an element of the blood-brain barrier and fingerlike projections that interpose between pre- and post-synaptic membranes to form the tripartite synapse. Electron micrographs have identified several unique ultrastructural features, including large mitochondrial length and prevalent stacks of endoplasmic reticulum (ER). Little information is known during early brain development when astrocytes acquire these characteristics. We studied astrocyte cellular structure during early developmental stages when the brain is enlarging, expanding the vasculature, and neural inputs compete for synaptic targets. The medial nucleus of the trapezoid body (MNTB) is a useful model system for studying neural circuit development due to the rapid maturation of a nearly homogenous neuronal population and its transition from multi-innervation to mono-innervation by the largest terminal in the mammalian brain, the calyx of Held (CH). We utilized our unique developmental series of serial block-face scanning electron microscopy (SBEM) image volumes to observe the change in the astrocyte morphology. We have completed the first full reconstruction of astrocytes at postnatal day (P)2, before CH growth, P4 and P6, during rapid growth and competition, and P9, when mono-innervation is likely complete. Qualitative comparison of the reconstructions reveals the presence of endfeet and contacts with nerve terminals from the earliest age. Quantification of these features showed the P2, P4, P6, and P9 astrocytes formed 1, 3, 2, and 1 endfoot respectively. We made the novel observation that large areas of MNTB cell bodies were covered with vellus (sheet-like) processes at all ages. Each astrocyte formed the vellus processes on multiple cells (5, 6, 7, 6 cells, respectively). After the CH began to grow, vellus processes covered its non-synaptic surface (P4, 6, 9). We observed the large CH axons wrapped by astrocytic processes at P4 and P6. CH axons are myelinated by P9, yet the P9 astrocyte extended processes that wrapped around the myelin. We skeletonized the cells to observe branching patterns and showed 3-4 primary processes at every age. In all cells, the nucleus was eccentric and had a lobular shape. From P2 onward, we observed the ultrastructural features of large mitochondrial length and stacks of ER found in mature astrocytes. Thus, several mature features of astrocytes were evident during early development at a stage after initial neural contacts had formed but prior to growth of mature nerve terminals, and as the vasculature was expanding and the density of vessels was increasing.

Disclosures: E.M. Amick: None. **D. Heller:** None. **P.S. Holcomb:** None. **V. Moses:** None. **A. Gabbard:** None. **M. Attalla:** None. **M.H. Ellisman:** None. **G.A. Spirou:** E. Ownership Interest

(stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); syGlass.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH K00ES033033
BWF Postdoc Enrichment Program
Emory University HERCULES Center (P30 ES019776)

Title: Cell-type specific effects of lead (Pb) toxicity in the developing human brain

Authors: M. SAMPSON, S. SONSUKAR, A. KING, E. HILL, A. VOSS, S. SLOAN; Emory Univ., Atlanta, GA

Abstract: Prenatal exposures to environmental toxicants can interfere with nervous system development and lead to persistent changes in the brain. In this work we examine how early-life exposure to the heavy metal lead (Pb) affects the generation and maturation of different cell types—neurons and astrocytes—using primary fetal brain and human induced pluripotent stem cell-derived 3D organoids. Previous studies have largely focused on neuronal toxicity, but here we leverage the reductionist organoid system to study astrocytes, specialized glial cells that can play important roles in neurotoxicity. Astrocytes protect the brain from toxicants by participating in the blood brain barrier, regulating inflammation, and providing redox and metabolic support to other cells. Following 1 week of Pb exposure, organoids upregulated expression of metallothioneins, which are involved in metal homeostasis and buffering, as well as pentose phosphate pathway genes, which restore the electron-donor NADPH and the antioxidant glutathione. Several glial differentiation genes were downregulated, suggesting a possible inhibition of glial generation or maturation. To examine cell-specific transcriptional responses to chronic Pb, we performed 10X scRNA-Seq on organoids derived from 3 iPSC lines and exposed to Pb or control media for 3 weeks. We assigned cells to major classes including astrocytes, neurons, radial glia, proliferating cells. Differential expression analysis of the astrocyte cluster suggests a strong ER-stress response (HSPA5, HSP90B1, MANF, DNAJB9) and a possible reactive phenotype (GFAP, S100A10, VIM). Chronic Pb increased the ratio of neurons to astrocytes in organoids, and our future work will address whether this is related to increased neurogenesis, astrocyte cell death, or a suppression of gliogenesis/glial maturation. Overall, our data suggest that developmental Pb may influence the cellular balance of neurons and astrocytes in the developing brain, which could contribute to Pb-associated IQ and behavioral deficits in young adulthood.

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Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.05

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH 5R01MH119156
NINDS R01NS102228

Title: Developmental origins and trajectories of heterogeneous astrocytes in the septum

Authors: *Y. XIE[1], A. A. GRANADOS[2], A. O. PISCO[2], C. M. REID[3], M. TURRERO GARCIA[1], M. ADAM[1], O. MOSTO[3], C. C. HARWELL[1], S. M. HANSON[1];
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Abstract: The septum is a ventral forebrain structure that is composed of a diversity of GABAergic and cholinergic neurons involved in the regulation of numerous innate behaviors. Astrocytes, a major glial cell type found throughout the central nervous system, perform a broad range of functions that influence the formation and activity of neural circuits, including regulating ion homeostasis, neurotransmitter reuptake and synaptic pruning. Emerging evidence shows that astrocytes acquire specialized molecular and functional features to suit the brain regions and circuits they support. The extent of astrocyte diversity in the septum and how different types of septal astrocytes acquire their unique properties are not understood. We used mouse genetic fate mapping to show that astrocytes in the septum with a developmental history of *Nkx2.1* or *Zic4* expression occupy the medial (MS) or lateral (LS) septum respectively, suggesting distinct developmental origins. To assess the molecular specification of septal astrocytes we performed single-nucleus RNA sequencing (snRNA-seq) at a series of postnatal developmental stages in the septum, distinguishing astrocytes derived from *Nkx2.1*-expressing versus non-*Nkx2.1*-expressing progenitors. Our analysis identifies two major transcriptionally distinct astrocyte subtypes that emerge over early postnatal time in the septum and three immature astrocyte subgroups. Validation of our data by fluorescent *in situ* hybridization revealed that those two major astrocyte populations occupy distinct spatial positions, located either in the medial or lateral septum. Our further cell-cell interactions analysis shows the interactions between neuron-astrocyte subtypes are enriched in distinct developmental signaling pathways, implying that both developmental origins and cellular environment contribute to the acquisition of unique features of each astrocyte subtype. This study provides a foundation for assessing the factors/mechanisms that contribute to the diversification of astrocytes to support specialized septal circuits.

Disclosures: **Y. Xie:** None. **A. A. Granados:** None. **A. O. Pisco:** None. **C. M. Reid:** None. **M. Turrero Garcia:** None. **M. Adam:** None. **O. Mosto:** None. **C. C. Harwell:** None. **S. M. Hanson:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH U01MH122590

Title: Novel approach to isolate oligodendrocyte precursor cell nuclei from human postmortem brain facilitated uncovering the epigenetic regulation in oligodendrocyte lineage

Authors: *A. KOZLENKOV[1], R. VADUKAPURAM[1], P. ZHOU[1], M. WEGNER[2], S. DRACHEVA[1,3];
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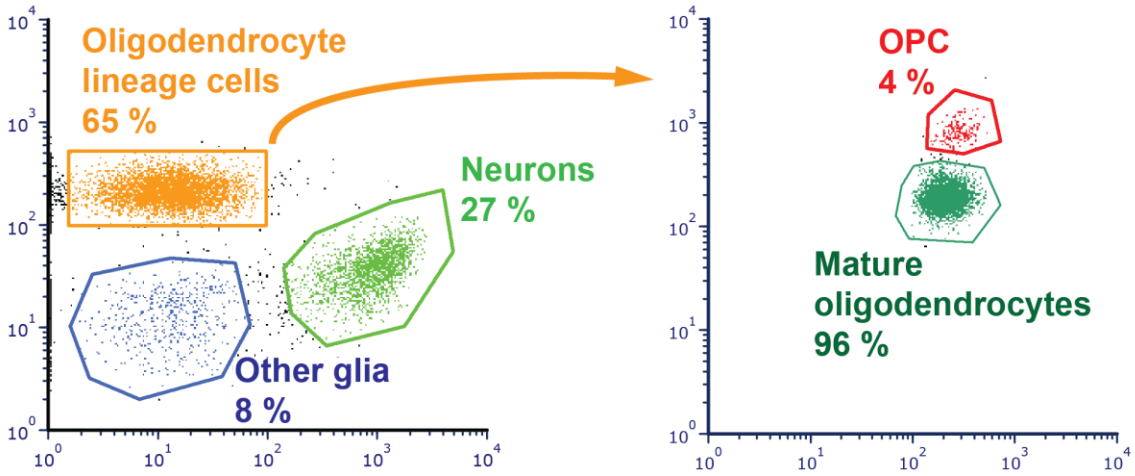
Abstract: Oligodendrocyte precursor cells (OPCs) are a subset of glial cells, whose main function is to generate myelinating mature oligodendrocytes (MOs) during development. OPCs also serve to replenish the pool of MOs in adult brain in adaptive plasticity, in neurodegenerative disorders, and after trauma. Whereas OPCs persist in the adult brain, it is well documented that their ability to differentiate and give rise to functional MOs decreases with age and may be specifically compromised in certain diseases. An important avenue to uncover the underlying mechanisms of these age-dependent changes in OPC function would be to study the epigenetic regulatory landscapes in OPCs and MOs as a function of development.
To this aim, we developed a novel flow-cytometry-based multi-color approach to isolate nuclei of OPC and non-OPC MO cells from human postmortem tissue (see Figure). We generated in-depth transcriptomic profiles of OPCs and MOs from adult brain, confirming the identities and purity of the isolated populations. We then used ChIP-seq to characterize the H3K27ac histone modification profiles (indicative of active promoters and enhancers) in these two populations. We detected remarkable differences between OPC and MO epigenomes, with up to a half of all regulatory regions being differentially acetylated between the two cell types. Notably, there was a clear concordance between the gene expression and epigenetic landscapes in the two cell types. We then applied our method to isolate OPCs from human postmortem brain samples from 0–2 year-old subjects, detecting a significantly higher proportion of OPC cells than in adult brain. We compared the regulatory landscapes between infant and adult brain and detected numerous developmental epigenetic changes in OPC or MO cells. Many of these changes were cell-type-specific, and included genes involved in cell cycle, protein synthesis, and neurotransmission. The

presented experimental approach and the uncovered epigenetic changes justify further in-depth studies of the regulatory landscapes of OPC and MO cells in human development and disease.



Disclosures: **A. Kozlenkov:** None. **R. Vadukapuram:** None. **P. Zhou:** None. **M. Wegner:** None. **S. Dracheva:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

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Program #/Poster #: 599.07

Topic: A.01. Neurogenesis and Gliogenesis

Support: DST INSPIRE Faculty fellowship (IFA-18-LSBM- 210)
TIFR DAE (12-R&D-TFR-5.10-0100RTI2001)
DST/CSRI/2-17/202

Title: Lhx2 regulates astrogliogenesis during development

Authors: ***A. IYER**, R. FRONTEIRO, P. BHATIA, A. SRIVASTAVA, S. TOLE;
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Abstract: Cortical astrocytes arise from radial glia, subventricular zone progenitors and by local proliferation of astroglioblasts. The molecular mechanisms governing astrogliogenesis are poorly understood. Transcription factor LHX2 is expressed abundantly in young and mature astrocytes in the mouse and human brain. We tested whether LHX2 is necessary for the generation of astrocytes in the mouse. Since astrogliogenesis peaks postnatally, we used hGFAP CreERT2 which is active in progenitors as well as differentiated astrocytes of the neocortex and the

hippocampus, and administered tamoxifen at postnatal day (P)1 to induce loss of LHX2 function (LHX2 LOF). The recombined cells were tracked using an Ai9 reporter background. LHX2 LOF caused a significant increase in the number of astrocytes in the neocortex and the hippocampus, which persisted into adulthood. While Lhx2 LOF astrocytes were spread across the entire cortex leaving few gaps, their distribution was not homogeneous, displaying regions of apparent clumping. This raised the possibility that the tiling mechanism and contact inhibition might be altered in these mutants, which is a part of the ongoing analysis. Proliferation marker analysis indicated that the increase in astrocyte number was due to enhanced proliferation. We used an alternative strategy, *in vivo* electroporation of the neonatal brain, which preferentially targets progenitors near and around the VZ and spares differentiated astrocytes that have migrated into the cortical plate. LHX2 LOF by this procedure also produced a significant increase in astrocytes. Together, these results suggest that LHX2 functions to control the number of astrocytes by suppressing their proliferation. In ongoing studies, we are examining the functional consequence of the enhanced astrogliogenesis, testing the hypothesis that it is protective in seizure-induced damage.

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Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.08

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS094171
NIH R01 NS105638

Title: Robo functions as an attractive cue for glial migration through SYG-1/Neph

Authors: *Z. QU, A. ZHANG, D. YAN;
Duke Univ., Duke Univ., Durham, NC

Abstract: As one of the most-studied receptors, Robo plays functions in many biological processes, and its functions highly depend on Slit, the ligand of Robo. Here we uncover a Slit independent role of Robo in glial migration and show that neurons can release an extracellular fragment of Robo upon cleavage to attract glia during migration in *Caenorhabditis elegans*. Furthermore, we identified the conserved cell adhesion molecule SYG-1/Neph as a receptor for the cleaved extracellular Robo fragment to mediate glial migration and SYG-1/Neph functions through regulation of the WAVE complex. Our studies reveal a previously unknown Slit-independent function and regulatory mechanism of Robo and show that the cleaved extracellular fragment of Robo can function as a ligand for SYG-1/Neph to guide glial migration. As Robo, the cleaved region of Robo, and SYG-1/Neph are all highly conserved across the animal

kingdom, our findings may present a conserved Slit-independent Robo mechanism during brain development.

Disclosures: Z. Qu: None. **A. Zhang:** None. **D. Yan:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.09

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS10499
NIH Grant T34GM127184

Title: Expression of Neurogenic Transcription Factors Redirect Astrocyte Fates of Postnatal Radial Glia in Mouse Neocortex.

Authors: *V. R. CUEVAS[1], J. J. LOTURCO[2];
[2]Physiol. and Neurobio., [1]Univ. of Connecticut, Storrs, CT

Abstract: Radial glial cells (RGCs) are multipotent neural progenitors that generate different cell types over the course of brain development. In the cerebral cortex, RGCs initially produce pyramidal neurons and later become restricted to generating astrocytes, oligodendrocyte precursors and ependymal cells. In this study we tested whether induced expression of any of seven transcription factors (Lhx2, Brn2, Neurod1, Lbx1, Myt1l, Ngn2, or Ascl1) in early postnatal (P0-P2) radial glia could alter the fates of cells generated from postnatal RGCs. We found that while none of the transcription factors tested increased the number of neurons generated from RGCs, several of the transcription factors, including Myt1l, Brn2, Ascl1, and Lhx2, significantly increased the number of astrocytes generated from RGCs. In addition, the transcription factors Lhx2, Brn2, or Neurod1 differentially increased the number of cortical plate astrocytes relative to white matter astrocytes. In controls, postnatal RGCs primarily generated white matter astrocytes with approximately 75% in white matter and 25% in the cortical plate. Expression of Lhx2, Brn2, or Neurod1 significantly shifted this distribution, such that approximately 75% of astrocytes generated by postnatal RGCs were located in layer 1 or the cortical plate and 25% of astrocytes were within the white matter. In addition to increasing the numbers of astrocytes generated by RGCs and changing their distribution, the transcription factor Myt1l uniquely resulted in a significant increase of a population of small undifferentiated cells with glial-like morphologies. In summation, our results indicate that postnatal RGCs can be redirected to produce more astrocytes by expression of several neurogenic transcription factors without simultaneously re-programming RGCs to produce neurons.

Disclosures: V.R. Cuevas: None. **J.J. LoTurco:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.10

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS120746

Title: Dna methylation regulates trkb isoform expression in cns

Authors: *X. WEI[1], M. L. OLSEN[2];
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Abstract: Tropomyosin receptor kinase B (TrkB), the canonical receptor of Brain-Derived Neurotrophic Factor (BDNF), is broadly expressed in developing and mature brain. BDNF/TrkB signaling is critical in central nervous system (CNS) development, neuronal cell growth, differentiation, synaptogenesis and synaptic plasticity, while dysregulated BDNF/TrkB signaling is implicated in a growing list of neurodevelopmental and neuropathological disorders. TrkB is encoded by the *Ntrk2*, a complex gene approximately 330kb in length. TrkB has two main isoforms expressed in the CNS, the full length TrkB (TrkB.FL) receptor and the truncated TrkB (TrkB.T1) receptor. We recently demonstrated that TrkB.T1 is the predominant isoform in the rodent cortex, is almost exclusively expressed in astrocytes, and appears critical for astrocyte morphological maturation. In contrast TrkB.FL is the main isoform expressed in neurons. Given the critical role for this signaling pathway in healthy brain development and mature CNS function, we aim to identify the molecular underpinnings for the cell type specific expression of each TrkB isoform. Our preliminary data indicates cell-type specific isoform expression is in part due to differential methylation patterns of the *Ntrk2* in astrocyte and neurons. We isolated astrocytes and neurons from p28 WT male mice cortex with magnetic isolation method and high molecular weight DNA was extracted to construct libraries for three biological replicates of astrocytes and neurons (microglial were used as a control). Nanopore sequencing was used to quantitatively assess the methylation status of the *Ntrk2* in its entirety. We identified marked differences in neuronal and astrocyte methylation. Notably, the majority of differentially methylated sites (DMSs) between astrocytes and neurons were found in the intron located after the first coding exon expressed in both TrkB.FL and TrkB.T1 and in regions which flank a unique TrkB.T1 exon, which indicated that DNA methylation may regulate TrkB isoform expression. Future work is aimed at investigating and manipulating these unique differentially methylated regions *in vitro* and *in vivo* to determine the role of DNA methylation patterns in *Ntrk2* cell-type specific isoform expression.

Disclosures: X. Wei: None. **M.L. Olsen:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.11

Topic: A.01. Neurogenesis and Gliogenesis

Support: T32NS115656
R01HL139712
R01HL146670
W81XWH2010199

Title: Histone-3 Lysine-4 Methylation and Neonatal White Matter Injury in Congenital Heart Disease

Authors: *M. STRAUSS, N. BALLÓN, A. KALYAN, N. ISHIBASHI;
Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC

Abstract: Advances in surgical techniques and therapeutic interventions have significantly reduced mortality rates in infants with congenital heart disease (CHD). Unfortunately, many of these children subsequently display a range of neurological disabilities, including deficits in motor, cognitive, and executive functioning. Magnetic resonance imaging (MRI) studies have revealed a high frequency of white matter (WM) injury (25-55%) in this population. Controlled modulation of epigenetic mechanisms is critical to proper WM development, and genetic mutations to enzymes which regulate these processes appear frequently in the CHD population. Specifically, *de novo* mutations have been identified to both a methyltransferase (KMT2D) as well as a demethylase (KDM5A) which mediate histone-3 lysine-4 (H3K4) methylation (me). As H3K4me is associated with learning and memory capabilities, these mutations represent a potential mechanistic basis for the neurological disabilities identified in the CHD population. Studies have provided evidence that immature WM is particularly vulnerable before birth. This lack of oxygenation has been shown to predispose the developing brain to diffuse WM injury, as OLs are highly susceptible to hypoxia-induced stress. Furthermore, recent evidence demonstrates that hypoxia may alter H3K4me. Taken together, these findings support the overarching hypothesis that genetic predisposition, in combination with a hypoxic environment, leads to abnormal H3K4me, resulting in OL/WM deficits in CHD. To investigate this hypothesis PDGFRα-cre::ROSA^{fsTRAP} mice were crossed with either KMT2D$^{flox/flox}$ or KDM5A$^{flox/flox}$ mice which allow conditional knocked-out (k/o) specifically in oligodendrocyte (OL) progenitor cells (OPCs). The effects of these k/o's on H3K4me and subsequent gene expression are being examined through both CHIP-seq and TRAP-seq approaches, followed by immunohistochemical analyses of OPC development, maturation, and myelination. To assess if the negative impacts of these mutations are preventable/reversible, OPCs from these mice, and from human induced pluripotent stem cells expressing mutations to these enzymes, will be cultured in the presence of small molecules which aim to correct altered H3K4me. The proposed studies will provide critical mechanistic information of the interaction between intrinsic genetic predisposition and environmental factors associated with CHD-induced neurological deficits. In addition, due to the opposing actions of the proposed mutations, this study will demonstrate the need for patient-specific treatment in the CHD population.

Disclosures: **M. Strauss:** None. **N. Ballón:** None. **A. Kalyan:** None. **N. Ishibashi:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.12

Title: WITHDRAWN

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.13

Topic: A.01. Neurogenesis and Gliogenesis

Support: 5R01NS102237-05

Title: Elucidating the upstream regulators of astrocyte maturation using in vivo genome-wide profile of histone modifications

Authors: *L. NAGENDREN[1], K. SAKERS[1], D. IRALA[1], Y. KOBAYASHI[1], P. TATA[1], C. EROGLU[1,2,3,4];
[1]Cell Biol., [2]Neurobio., Duke Univ. Med. Ctr., Durham, NC; [3]Duke Inst. for Brain Sci. (DIBS), Durham, NC; [4]Howard Hughes Med. Inst., Durham, NC

Abstract: Complex morphological changes acquired by astrocytes during postnatal maturation are crucial for their functions at the synapse. However, the determinants that drive astrocyte maturation are not elucidated. While epigenetic modifications control the gliogenic switch in neural stem cells, the role of epigenetic changes remains unexplored in directing astrocyte maturation postnatally. In this study, we chose to focus on histone modifications due to the prevalence of histone-modifying enzymes in neurodevelopmental diseases, such as Autism. Further, astrocytes have higher expression of many of these enzymes compared to neurons. We profiled the dynamics of histone modifications in mouse cortical astrocytes across postnatal development and used this data to elucidate how chromatin states change. **M**ultiplexed **In**dexed **T**7 **Ch**romatin **I**mmuno**p**recipitation sequencing (Mint-ChIP) was performed on mouse cortical astrocyte nuclei collected from both sexes at different developmental time points: postnatal days, P7, P14, P21, and P150. Mint-ChIP allows for immunoprecipitation and quantification of six histone modifications across the genome. We profiled H3K4me1, H3K4me3, H3K27ac, H3K36me3, H3K27me3, and H3K9me3 and quantified their abundance compared to total H3. ChromHMM was used to characterize the chromatin states (i.e., active transcription start sites

(TSS), repressed polycomb) based on the histone modifications. To investigate the upstream regulators of astrocyte maturation, we profiled transcription factor (TF) binding motifs in epigenetically-defined active TSSs across time points. We identified four TFs with high inclusion of the binding motifs at P7 but mostly absent in later time-points. To understand the functions of the genes containing the binding motifs of a given TF, we performed a Gene Ontology analysis which showed that these genes are highly involved in morphological maturation and synaptogenesis, such as axon guidance and regulation of neuron projection development. To further test the significance of the temporal expression of these TFs in cortical astrocytes, we are utilizing the CRISPR-Cas9 system to knockout TFs of interest and quantify the astrocyte morphological maturation using Imaris. Together, our data lays the foundation for elucidating astrocyte epigenetic landscape dynamics in vivo. Using these data, we identified and tested TFs that are important postnatally for astrocyte maturation. These data are useful for understanding how mutations in histone-modifying enzymes may result in epigenetic changes that alter astrocyte development.

Disclosures: **L. Nagendren:** None. **K. Sakers:** None. **D. Irala:** None. **Y. Kobayashi:** None. **P. Tata:** None. **C. Eroglu:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.14

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 8P20GM103346

Title: Maternal helminths in rats alter microglia development within the first week of postnatal life

Authors: *E. T. MATTHEWS[1], L. L. WILLIAMSON[2];
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Abstract: Early-life inflammation can have prolonged effects on cognitive function. Microglia, often the main sources of neuroinflammation, proliferate and develop rapidly during the first 2 weeks of postnatal life. To determine if the effects of neonatal inflammation could be attenuated by *in utero* and postnatal maternal environments, female Sprague-Dawley rats were treated orally with *Hymenolepis diminuta* (rat tapeworms) or saline, prior to conception. We collected brain tissue from 1 male and 1 female per litter on postnatal day one (P1) and P4. The remaining pups in the litter received an injection of *E. coli* or PBS and then we collected 1 male and 1 female per litter on P7. Brain slices (20 micron) were mounted on slides and stained with Iba1 antibody. Using unbiased stereology, hippocampal microglia were categorized based on the processes present (i.e., amoeboid, stout, thick long, thin ramified). In P1 brains, there was a large presence of amoeboid and stout microglia, there were few thick long and they lacked thin

ramified cells. With the P4 pups there was a large decrease of amoeboid microglia and an increase in all other morphologies, indicating maturation. When comparing the most mature morphology of each age group - thick long cells on P1 and thin long cells on P4, pups born to dams that received helminths have fewer mature cells compared to saline treatment. We predict that these differences in maturation will reach equilibrium by P7. These findings will elucidate the effects of maternal parasite infection on microglia development in the early postnatal period.

Disclosures: E.T. Matthews: None. **L.L. Williamson:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.15

Topic: A.01. Neurogenesis and Gliogenesis

Support: Cancer League of Colorado AWD-220552-SF

Title: Developing a novel mouse model using in uteroelectroporation to study pediatric high grade glioma

Authors: *M. MASON, J. DESISTO, S. MITRA, A. GREEN, S. FRANCO;
Univ. of Colorado Anschutz, Aurora, CO

Abstract: Pediatric high-grade gliomas are a set of aggressive childhood brain tumors that respond poorly to current cancer therapies. High-grade pediatric diffuse midline gliomas (DMGs) often have a notable absence of a myeloid or lymphoid infiltrate that fails to correlate with survival. This makes DMG a unique tumor entity that appears not to have active immune evasion or suppression mechanisms and does not respond to the current generation of immunotherapies. I hypothesize that because DMGs develop concurrently with the immune system, they are recognized as a “normal” self-tissue. To begin to test this hypothesis, I sought to develop a mouse model of DMG that mimics the co-development of tumors and the immune system. Using *in utero* electroporation (IUE) on CD-1 mice of both sexes, I expressed genes known to be involved in DMGs by using a combination of mutated histone H3.3 K27M, dominant-negative TP53, and constitutively active PDGFRA. I specifically electroporated neural progenitors in the ventricular zone of the 4^{th} ventricle to target the developing pons. I then dissected the brainstem at postnatal days 7, 14, 21, 35, and 42 and performed histological analysis to determine tumor grade, as well as immunohistochemistry for various DMG and cell-type markers. Preliminary findings indicate that oncogene expression causes defects in cell growth, morphology, gene expression, and ultimately DMGs when compared to a control. Interestingly, IUE of the oncogenes, but not control plasmids, appeared to cause an increase in the numbers of microglia in the electroporated region just a few days after electroporation. Our next step is to better characterize all stages of tumor development and the tumor-immune environment in the IUE model. Further development of this model is vital towards better

understanding the developmental origins of pediatric gliomas and the mechanisms regulating tumor-immune cell interactions, and for developing personalized therapies to treat this devastating disease.

Disclosures: **M. Mason:** None. **J. Desisto:** None. **S. Mitra:** None. **A. Green:** None. **S. Franco:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.16

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NINDS R01 NS109239

Title: Shh signaling and Ascl1 promote oligodendrogenesis in dorsal forebrain neural progenitor cells

Authors: *A. BALOLIA, C. WINKLER, M. MASON, S. FRANCO;
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Abstract: Oligodendrocytes are glial cells that wrap neuronal axons with myelin to enable faster signal transduction and promote axon health. Failure of oligodendrocytes to properly form or maintain myelin gives rise to a variety of neurological dysfunctions, including motor impairments, learning disabilities and psychiatric disorders. Thus, elucidating the mechanisms that contribute to the development and maintenance of oligodendrocytes is an essential step towards a more complete understanding of brain development and disease. Although it is known that oligodendrocytes in the neocortex are largely produced by neural progenitor cells (NPCs) located in the dorsal forebrain, the precise mechanisms governing NPC fate are not fully understood. We previously showed that deletion of *Smo*, the Sonic Hedgehog (Shh) signaling effector, significantly reduces the number of oligodendrocytes generated by NPCs in the embryonic neocortex, indicating that Shh signaling is important for oligodendrocyte fate. Additionally, single-cell mRNA sequencing of dorsal forebrain NPCs has implicated the transcription factor Ascl1 in the generation of oligodendrocyte precursor cells (OPCs). I hypothesize that Ascl1 and Shh signaling synergize to drive NPCs towards an oligodendrocyte fate. To examine this relationship, I studied the effects of Ascl1 loss- and gain-of-function in Shh-mediated OPC genesis during embryonic and early postnatal forebrain development. Mouse embryos were electroporated *in utero* with cDNA and CRISPR vectors at embryonic day 15.5 and analyzed 1, 3, or 15 days later. Brains were sectioned and immunoassayed for two markers of the oligodendrocyte lineage: the PDGFRα surface receptor and the Olig2 transcription factor. My preliminary studies indicate that progenitors overexpressing Ascl1 together with constitutively-activate Smo (SmoA1) produced more OPCs compared to their counterparts expressing SmoA1 alone. Conversely, CRISPR-mediated knockout of *Ascl1* blocked the

SmoA1-induced increase in OPCs. Future studies will focus on the molecular mechanisms by which Ascl1 cooperates with Shh signaling to specify the oligodendrocyte lineage.

Disclosures: **A. Balolia:** None. **C. Winkler:** None. **M. Mason:** None. **S. Franco:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.17

Topic: B.09. Glial Mechanisms

Support: Cure Alzheimer's Fund (CAF)
The Alzheimer's Association
Parekh Center (NYU)
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NIH Grant 5RM1HG009491 (JDB)
NIH Grant 3RM1HG009491-03S1 (JDB/SAL)
NIH Grant 3RM- 1HG009491-03S2 (JDB/SAL)

Title: Single-cell analysis of early differentiated glia in human and mouse

Authors: *P. FRAZEL[1], D. LABIB[3], A. MARCHILDON[1], R. BROSH[2], J. BOEKE[2], V. FOSSATI[3], S. A. LIDDELOW[1];
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Abstract: Central nervous system macroglia (astrocytes and oligodendrocytes) are required for normal brain development and function, and are among the last cells to emerge during neurodevelopment. Many questions remain about their emergence in the brain and spinal cord, including how early glial fates are specified during development or differentiation, and similarly when subtypes of glia are specified. Here, we used single-cell RNA sequencing (scRNAseq) to analyze 100,000+ cells across multiple timepoints during the differentiation of astrocytes and oligodendrocytes. We analyzed glial differentiations originating from both human induced pluripotent stem cells and mouse embryonic stem cells. Using time series analysis of gene expression (Waddington Optimal Transport algorithm), we uncovered multiple genes involved in fate specification of glial subtypes in both species. We examined gene expression changes during intermediate states of glial specification, and were able to identify genes that were correlated with the choice between neuron versus glia in both species. Using our scRNAseq data, we optimized previous mouse astrocyte differentiation protocols by highlighting and removing non-required transition states and decreasing the overall protocol from 3 weeks to less than 12 days. We also compared tandem chromatin accessibility and gene expression (10X Genomics multiome) at the single-cell level for later timepoints in the mouse differentiation to compare *in vitro* chromatin changes with published mouse glial development data. Our data will be useful

for researchers interested in optimizing glial differentiations in either species, and further provide a window into human glial differentiation, which is difficult to study given its occurrence late in development.

Disclosures: P. Frazel: None. **D. Labib:** None. **A. Marchildon:** None. **R. Brosh:** None. **J. Boeke:** None. **V. Fossati:** None. **S.A. Liddelow:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.18

Title: WITHDRAWN

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.19

Topic: B.09. Glial Mechanisms

Title: Expression of glutamate transporters GLAST and GLT-1 in three echinoderms of the Mexican Pacific

Authors: *T. OLIVARES-BAÑUELOS[1], A. COLORES-MENDOZA[1], F. CORREA-SANDOVAL[1], L. ENRÍQUEZ-PAREDES[2], A. ORTEGA[3];
[1]Inst. de Investigaciones Oceanológicas, [2]Facultad de Ciencias Marinas, Univ. Autónoma de Baja California, Ensenada, Mexico; [3]Toxicología, Cinvestav-IPN, Mexico City, Mexico

Abstract: Marine invertebrates are strategic models for neurosciences. Invertebrates and vertebrates nervous systems are mainly made up of neurons and glia cells, which have similarities in their biochemistry and functionality. Presences of glial cells in few echinoderm species have been demonstrated, and we previously reported gene expression of glutamate transporter GLAST in larvae and post-larvae of the Mexican Pacific sea biscuit *Dendraster excentricus*. Now we analyzed the expression of GLAST and GLT-1 glutamate transporter in 1) larval, post-larvae, and adult tissue of the *D. excentricus*, 2) in adult tissue of the red sea urchin *Strongylocentrotus franciscanus* and 3) in adult tissue of the sea cucumber *Isostichopus fuscus*. Echinoderm adult organisms were collected from the Punta Banda bay in Baja California, Mexico. *Dendraster excentricus* larvae and post-larvae cultures were carried in the laboratory. Quantitative PCR was carried out by triplicate to measure *GLAST* and *GLT-1* gene expression; *Ribosomal 18S*, *Actin*, and *Ferritine* housekeeping genes expression were measured too as

internal controls. Sequencing of expressed glutamate transporters genes was done to verify the amplicon. Results showed statistically significant differences in GLAST expression between adults, larvae and post-larvae of *D. excentricus*. As expected, adult echinoderms presented the higher relative expression of GLAST and GLT-1 genes. Results showed us that functional glia cells are present in adults of *D. excentricus*, *S. franciscanus*, and *I. fuscus*. These results and the fact that echinoderms have a much simpler nervous system than chordates, despite their close phylogenetic relationship, allow these marine organisms to be used for the study of neurodegenerative diseases and their possible treatment.

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Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.20

Topic: B.09. Glial Mechanisms

Support: National Science Foundation Grant No. IOS-1921065
National Institutes of Health, NINDS R01NS118562

Title: Identification of astrocyte subtypes in the Drosophila melanogaster visual system

Authors: *J. SAUNDERS[1], Y. Z. KURMANGALIYEV[3], A. D. R. GARCIA[2], C. R. VON REYN[1];
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Abstract: The diversity of glial cells, particularly astrocytes, has gained increasing recognition. Astrocytes are the most abundant glia cell type and regulate synapse formation, maturation, and function. Growing evidence from rodent studies shows a remarkable diversity and complexity of astrocyte subpopulations. In contrast, if astrocytes in *Drosophila melanogaster*comprise multiple subpopulations is unknown. *Drosophila melanogaster* is a good model for studying astrocyte diversity due to emerging connectomes and conserved astrocyte properties between vertebrates and invertebrates. Evidence for astrocyte subtypes in *Drosophila* comes from sc-RNAseq datasets in the developing visual system that identified 19 glial clusters (Kurmangaliyev 2020). An independent study in the adult visual system also identified 19 glial clusters (Özel 2021), suggesting similarities between transcriptionally defined glial populations in the pupal and adult brain. These findings have raised questions about possible diversity among the six well-defined glial classes in *Drosophila*. Here, we focus on identifying astrocyte populations among the 19 glial clusters in the pupal and adult brain of *Drosophila.* We establish a bioinformatics and immunohistochemistry pipeline to investigate astrocyte heterogeneity. We utilize three known

genetic markers to classify astrocytes among unlabeled glia clusters. The markers *alrm*, *Eaat1*, and *Gat* separate astrocytes among various glial populations in newly eclosed flies (96 hours after pupal formation (hAPF)), but only *Eaat1* and *Gat* reliably separate astrocytes during adulthood. Using these markers, we identify two putative astrocyte clusters in both datasets that are predicted to be astrocytes. A correlation analysis reveals the predicted astrocytes in each dataset have the highest correlations when comparing their genetic profiles. We then sought to identify genetic differences between the predicted astrocytes. A differential gene analysis identified 3 genes differentially expressed between predicted astrocytes at 96 hAPF and 30 genes differentially expressed between predicted adult astrocytes. Future work will examine protein expression and *in vivo* localization of these genes to determine whether clusters represent subtypes of astrocytes or astrocytes in different states. We will then use the bioinformatics pipeline to investigate other glia cell types in these datasets as we suspect them to exhibit similar diversity. Overall, this work provides insights for identifying diversity among glia populations and aids in the understanding on the mechanisms glia cells have in regulating neuronal connectivity.

Disclosures: J. Saunders: None. **Y.Z. Kurmangaliyev:** None. **A.D.R. Garcia:** None. **C.R. von Reyn:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.21

Topic: B.09. Glial Mechanisms

Support: 2021R1A2C1008317

Title: Phospholipase D1 promotes astrocytic differentiation through the FAK-AURKA-STAT3 signaling pathway in hippocampal neural stem progenitor cells

Authors: *S.-Y. PARK, M.-J. KANG, N. JIN, J.-S. HAN;
Hanyang University, Biomedicine Inst., Hanyang University, Biomedicine Inst., Seoul, Korea, Republic of

Abstract: Phospholipase D1 promotes astrocytic differentiation through the FAK-AURKA-STAT3 signaling pathway in hippocampal neural stem/progenitor cells
Shin-Young Park, Min-Jeong Kang, Nuri Jin and Joong-Soo Han
Biomedical Research Institute and Department of Biochemistry and Molecular Biology, College of Medicine, Hanyang University, Seoul 04763, Republic of Korea
Phospholipase D1 (PLD1) plays a crucial role in cell differentiation of different cell types. However, the involvement of PLD1 in astrocytic differentiation remains uncertain. In the present study, we investigate the possible role of PLD1 and its product phosphatidic acid (PA) in astrocytic differentiation of hippocampal neural stem/progenitor cells (NSPCs) from hippocampi

of embryonic day 16.5 rat embryos. We showed that overexpression of PLD1 increased the expression level of glial fibrillary acidic protein (GFAP), an astrocyte marker, and the number of GFAP-positive cells. Knockdown of PLD1 by transfection with Pld1 shRNA inhibited astrocytic differentiation. Moreover, PLD1 deletion (*Pld1*$^{-/-}$) suppressed the level of GFAP in the mouse hippocampus. These results indicate that PLD1 plays a crucial role in regulating astrocytic differentiation in hippocampal NSPCs. Interestingly, PA itself was sufficient to promote astrocytic differentiation. PA-induced GFAP expression was decreased by inhibition of signal transducer and activation of transcription 3 (STAT3) using siRNA. Furthermore, PA-induced STAT3 activation and astrocytic differentiation were regulated by the focal adhesion kinase (FAK)/aurora kinase A (AURKA) pathway. Taken together, our findings suggest that PLD1 is an important modulator of astrocytic differentiation in hippocampal NSPCs via the FAK-AURKA-STAT3 signaling pathway.

Disclosures: S. Park: None. **M. Kang:** None. **N. Jin:** None. **J. Han:** None.

Poster

599. Mechanisms of Glial Development

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 599.22

Topic: B.09. Glial Mechanisms

Support: Adelson Medical Research Foundation
Sana Biotechnology

Title: In vivo context-dependent differentiation of transplanted human glial progenitor cells

Authors: *J. N. MARIANI, S. J. SCHANZ, C. C. LONG, K. MCCOY, D. CHANDLER-MILITELLO, S. A. GOLDMAN;
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Abstract: Neither rodent models nor in vitro studies of human cells fully reflect the molecular regulation of human glial progenitor cell (hGPC) expansion, differentiation and myelination in vivo. To this end, we used scRNA-seq to characterize pluripotent stem cells and their derived hGPCs, as produced from either hESCs (WA09) or iPSCs (C27), at three different stages: as undifferentiated stem cells, prior to neural or glial induction; following their in vitro induction and differentiation as hGPCs; and 19-20 weeks after the neonatal transplantation of these hGPCs into immunodeficient and myelin-deficient shiverer mice. Differentiated hGPCs were transcriptionally non-overlapping with their parental undifferentiated PSCs; we were unable to identify any residual undifferentiated cells in hGPC cultures after their glial differentiation, a process that spanned >160 days in vitro. Instead, these hGPC stage cultures were comprised of GPCs, astrocytic lineage cells, and a minority of neural stem and progenitor cells, as well as those transitioning between these cell types. Strikingly, transplantation of these cells into shiverer mouse corpus callosum resulted in substantial context-dependent differentiation, such

that when the human cells were extracted back from the chimeric brains 19 weeks after neonatal transplant, they were found to be comprised primarily of well-differentiated astrocytes, oligodendrocytes and their direct progenitors, all characterized by transcriptional states more mature than those achieved in vitro, while the overall extracted human cell population was largely devoid of early neural and non-neural as well as pluripotency genes. We next sought to identify transcription factors (TFs) active in these fate-directed subpopulations, through a combination of gene co-expression, motif enrichment, and extrapolation over cell-trajectories. This technique appropriately identified several lineage-specific TFs, including SOX10 and THRA in oligodendrocytes, and SOX9 and HES1 in astrocytes. Through integration of these analyses, we generated a transcriptional network governing both early and late stages of myelination, which highlighted the potential network importance of the TFs TFEB and KLF13. By analyzing over 40,000 cells, we have identified pathways whose targeting may permit the therapeutic modulation of glial specification, expansion, and terminal maturation of resident and transplanted human glial progenitor cells.

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Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.01

Topic: A.03. Stem Cells and Reprogramming

Support: Department of Defense Grant W81XWH2110209

Title: Dual-color calcium imaging operates as a novel drug screening platform in iPSC-derived heterogeneous neuronal culture systems

Authors: *N. TEANEY, W. AFSHAR SABER, K. WINDEN, M. SAHIN;
Neurol., The Rosamund Stone Zander Translational Neurosci. Ctr. (RSZ TNC) - The F.M. Kirby Neurobio. Ctr. – Harvard Med. Sch. – Boston Children’s Hosp., Boston, MA

Abstract: Calcium imaging serves as a functional assay to measure single-cell neuronal activity and the connectivity of the neuronal networks. In neurological disorders where neuronal signaling and network activity are altered, calcium imaging in 2D and 3D *in vitro* models may provide further insight into understanding the underlying cellular mechanisms driving the disorders. Furthermore, calcium imaging has the potential to serve a drug screening platform. While multi-electrode arrays provide critical insight into the overall electrical activity of a complex heterogeneous cell culture system, there is low spatial resolution in the readout (Gross et al., 1995). To our knowledge, there have been no methods developed to measure the functional activity of a heterogeneous population of cell culture system at a single-cell

resolution. In the present study, we demonstrate the feasibility of measuring the functional activity of two distinct populations of neurons derived from induced pluripotent stem cells (iPSCs) in a 2D co-culture system in a method described as *dual-color calcium imaging*. The method includes co-culturing NGN2 (Zhang et al., 2013) and iGABA (Yang et al., 2017) control GON0515-03 #5 iPSC-derived neurons after transducing each population with a separate genetic encoded calcium indicator (GECI). A GFP-based and an mRuby-based GECI are specifically chosen so that the excitation/emission spectra did not overlap with one another (excitation/emission of hSynl-GCaMP6s: 485/510 nm (Akerboom et al., 2012); hSyn1-jRCaMP1b: 550/600nm (Dana et al., 2016)). Calcium imaging recordings are collected to measure the changes in functional activity of the iGABA and NGN2 neurons before, during, and after the addition of synaptic blockers. As a proof of concept, synaptic blockers are added to the co-culture system to elicit changes in neuronal activity in each neuron population within the co-culture. Such synaptic blockers include Vigabatrin, an irreversible inhibitor of GABA transaminase; CNQX, a competitive AMPA receptor antagonist; and DAP-V, a competitive NMDA receptor antagonist (Ben-Menachem, 2011; Honore et al., 1998; Davies et al., 1981). Continuous blue and green illumination excite the green-shifted and red-shifted GECIs, respectively, within the different sub-types of neurons. In dual-color calcium imaging, the use of distinct GECIs in a heterogeneous cell culture system provides a novel platform for single-cell resolution drug screening. Future work will include utilizing the dual-color calcium imaging screening platform in iPSC-derived disease models in which neuronal signaling and connectivity are altered.

Disclosures: **N. Teaney:** None. **W. Afshar Saber:** None. **K. Winden:** None. **M. Sahin:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH-NINDS Grant NS096282
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Title: Printing functional human neural tissues

Authors: ***Y. YAN**[1], X. LI[1], Y. GAO[1], S. MATHIVANAN[1], L. KONG[1], Y. TAO[1], Y. DONG[1], X. LI[1], A. BHATTACHARYYA[1,2], X. ZHAO[1,3], S.-C. ZHANG[1,3,4,5];
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Biol., [3]Dept. of Neurosci., [4]Dept. of Neurol., Univ. of Wisconsin-Madison, Madison, WI; [5]Program in Neurosci. and Behavioral Disorders, Duke-NUS Med. Sch., Singapore, Singapore

Abstract: Probing how the human neural networks operate is hindered by the lack of reliable human neural tissues amenable for dynamic functional assessment of neural circuits. We developed a 3D bioprinting platform to assemble tissues with defined neural cell types in a desired dimension. The printed neuronal progenitors differentiate to neurons and form functional neural circuits in and between tissue layers within weeks, evidenced by spontaneous synaptic currents and synaptic response to neuronal excitation. Printed astrocyte progenitors develop into mature astrocytes with elaborated processes and form functional neuron-astrocyte networks by calcium flux and glutamate uptake in response to neuronal excitation. These designed human neural tissues will likely be useful for understanding the wiring of human neural networks, modeling pathological processes, and serving as platforms for drug testing.

Disclosures: Y. Yan: None. **X. Li:** None. **Y. Gao:** None. **S. Mathivanan:** None. **L. Kong:** None. **Y. Tao:** None. **Y. Dong:** None. **X. Li:** None. **A. Bhattacharyya:** None. **X. Zhao:** None. **S. Zhang:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.03

Topic: A.03. Stem Cells and Reprogramming

Title: Pharmacological manipulation of human brain wave-like activity in human iPSC-3D cortical circuits

Authors: S. THEISS[1], J. ISZAK[2], ***S. ILLES**[2];
[1]Inst. of Clin. Neurosci., Univ. of Duesseldorf, Duesseldorf, Germany; [2]The Sahlgrenska Academy, Univ. of Gothenburg, Goeteborg, Sweden

Abstract: Human brain wave activity prominently exhibits oscillatory network events (0.5—100 Hz) generated from neuronal assemblies within the human brain parenchyma that emerge as early as during fetal brain development. The shape, power, and spectral composition of human brain wave activity relies on orchestrated communication between excitatory, inhibitory, and modulatory neurons, which depends on their excitability and neurotransmitter receptor function. Since rodent models have limited translatability to humans, and human fetal and adult brain tissues are not routinely accessible, we have utilized a human iPSC-derived 3D cortical aggregate *in vitro* model combined with microelectrode array (MEA) technology to assess the contribution of specific neurotransmitter receptor functions on oscillatory activity (0.5—100 Hz) in human neuronal circuits.
We demonstrate that in our human iPSC-derived 3D cortical aggregate *in vitro* model, oscillatory activity (0.5—100 Hz) emerges within a few weeks and depends on neuronal excitability and

synaptic communication. We show that oscillatory activity is abolished by blockage of voltage-gated sodium channels or inhibition of glutamatergic synaptic transmission. In detail, oscillatory activity and synchronous neuronal network burst events are strongly suppressed or abolished after applying AMPA- or NMDA-glutamate receptor antagonists. Inhibition of $GABA_A$-receptor function and Carbachol-induced activation of cholinergic receptors change oscillatory activity and synchronous neuronal network properties. Interestingly, in the presence of Carbachol, oscillatory activity and neuronal network bursting are sustained after blockage of AMPA-receptors but are abolished by blockage of NMDA-receptors. This modulatory functional phenotype is comparable with the Carbachol-induced oscillatory activity and neuronal network burst events in acute mouse cortex slice preparations.
We demonstrate that our human iPSC-derived 3D cortical aggregate *in vitro* model allows assessing the full spectra of human brain cell functionality within a few weeks and enables the assessment of compound effects on human brain wave-like oscillatory activities in human iPSC-cortical circuits.
See also poster *"The emergence and properties of human brain wave-like activity in human iPSC-3D cortical circuits".*

Disclosures: S. Theiss: None. **J. Iszak:** None. **S. Illes:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.04

Topic: A.03. Stem Cells and Reprogramming

Title: A molecular toolbox for rapid generation of conditionally mutant human stem cell-derived neuronal models

Authors: *B. MARCO DE LA CRUZ, S. MITRA, F. H. STERKY;
Univ. of Gothenburg, Göteborg, Sweden

Abstract: Over the past decades, next-generation sequencing technologies have broadened our understanding of brain disorders as dozens of genetic risk factors for neurological disease have been identified. Yet, our ability to functionally dissect the molecular impact of novel risk variants has remained limited, in part due to inaccessibility of human brain samples. Progress in the use of human pluripotent stem cell models (hPSC) combined with precise gene editing tools, have now provided unprecedented opportunities for probing the contribution of specific mutations in disease-relevant neuronal models on a human genetic background. A widespread strategy involves comparison of cells from separate clones, but clonal selection is inevitably associated with a risk of amplifying stochastic genetic variations. Moreover, some mutations may impair cell viability. A strategy to circumvent these challenges is to generate conditional knockout or knockin cell lines, in which the same clonally expanded cells can give rise to both control and mutant cells. Here, we developed an efficient conditional knockout strategy in hPSCs

using homologous recombination (HR) facilitated by electroporation of CRISPR-Cas9 ribonucleoproteins (RNP) coupled to adeno-associated virus AAV-DJ donor template delivery. To this end, we have designed and implemented a set of molecular building blocks that allow rapid single-step Golden-gate-based cloning of large repair templates and targeting vectors. Using this strategy and combining it with very rapid purification of AAV, two GFP-tagged genes (ACTB and LMNB1) were targeted in hPSC. The rates of HR assessed by FACS sorting for GFP+ AAV-transduced cells, showed a targeting efficiency rate of up to 80%. Finally, different neurodevelopmental disease associated genes were robustly edited and human induced cortical excitatory neurons were generated for phenotypic characterization. Our work provides a time-effective versatile approach to facilitate functional studies that will ultimately help gaining insights in the molecular pathophysiology of neurological disorders.

Disclosures: B. Marco de la Cruz: None. **S. Mitra:** None. **F.H. Sterky:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.05

Topic: A.03. Stem Cells and Reprogramming

Support: HI21C1212
2021R1A6A3A01087248
HU20C0187

Title: A novel organs-on-a-chip platform mimicking the brain to gut pathway via visceral motor neurons; Using patient-derived organoids for Alzheimer's disease

Authors: *S. HONG, H. CHOI, K. AHN, I. MOOK-JUNG;
Dept. of Biomed. Sci., Seoul Natl. University, Col. of Med., Seoul, Korea, Republic of

Abstract: Alzheimer’s disease (AD) is an neurodegenerative disease that causes the degeneration of cells in the brain and the cognitive decline associated with the formation of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs). Recently, the destruction of gut-brain axis (GBA) is well-known as one of several causes of the neurodegenerative diseases including AD. The brain and gut are affected each other through dynamic bidirectional communications. However, these processes were still unknown regarding exact mechanism or starting point (whether brain is firstly changed and it affects gut or vice versa). Our previous study showed that the differences of brain pathologies between wild type (WT) mouse and AD model mouse led to changes in the gut microbial communities in situation where diet and environments are controlled. These results suggested that the brain pathologies in AD can affect the gut environment including gut microbiota, which gives us motivation to study mechanism regarding brain to gut pathway. However, it is difficult to study the brain to gut pathway directly from human patients and animal models because of the complex physiology. To solve this

problem, we used the patient derived-induced pluripotent stem cells (iPSCs) and microfluidic device to study the brain to gut pathway. First, we cultured the brain organoids, vagus nerve and gut organoids derived from human iPSCs. In next step, to verify how the brain organoids in AD condition affect gut environments and which mechanisms work, we integrated the brain organoids, vagus nerve and gut organoids in the microfluidic chamber. Our findings suggest that gut dysbiosis and disturbance in gut environment are consequence of brain pathology rather than cause of AD progression. These organ-on-a-chip can provide important clues for comprehending complex mechanism of GBA in AD and it could be utilized drug screening system targeting GBA for AD patients.

Disclosures: S. Hong: None. **H. Choi:** None. **K. Ahn:** None. **I. Mook-Jung:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.06

Topic: A.03. Stem Cells and Reprogramming

Support: HU20C0187
2021R1A6A3A01087248
HI21C1212

Title: A novel organs-on-a-chip platform mimicking the gut to brain pathway via visceral sensory neurons; Using patient-derived organoids for Alzheimer's disease

Authors: *K. AHN, H. CHOI, S. HONG, I. MOOK-JUNG;
Dept. of Biomed. Sci., Seoul Natl. University, Col. of Med., Seoul, Korea, Republic of

Abstract: Numerous clinical and experimental data suggest that the gut-brain axis plays an essential role for the expression and progression of neurodegenerative disorders. Most of these previous efforts have explained these relationships by the gut-blood-brain axis, especially the dysbiosis of the immune system. Vagus nerve is also attracting attention as one of the important bridges among the gut-brain axis in neurodegenerative disease. Previous works about neurodegenerative diseases focused on the parasympathetic component of vagus nerve to examine the effects of the gut-brain axis. However, it is well known that visceral sensory neuron of the vagus nerve also has a major impact on gut-brain axis. Thus, our study intends to provide a unique method to investigate gut-visceral sensory nerve-brain axis via organs-on-a-chip model.Current organs-on-a-chip model connects colon organoids, brain organoids and induced-visceral sensory neuron produced from human-derived induced pluripotent stem cells (hiPSCs). As none of the previous works established protocols to generate visceral sensory neurons from hiPSCs, we set up a novel protocol to generate them. Generation of colon organoids and brain organoids followed previous established protocols.By producing the chip using Alzheimer's disease (AD) patient-derived hiPSCs, our data have shown that AD patient-derived colon

organoids could provoke AD pathology to non-AD patient-derived brain organoids.In conclusion, our platform provides a novel method which could shed a light for determining various impacts of visceral sensory neuron in neurodegenerative disease model. Moreover, our chip could be useful for investigating the role of visceral sensory neuron in various fields, such as appetite or nutrient sensing models in endocrinology.

Disclosures: K. Ahn: None. **H. Choi:** None. **S. Hong:** None. **I. Mook-Jung:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.07

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R21EY033127
CellSight Development Fund
Grant to the Department of Ophthalmology at the University of Colorado from Research to Prevent Blindness
Linda Crnic Institute for Down Syndrome

Title: Development of a Novel Human iPSC-Derived Organoid Model of Retinal Alzheimer's Disease Histopathology

Authors: *E. JAMES[1], A. VIELLE[1,3,2], H. LI[5], N. R. JOHNSON[2,3,4], H. J. CHIAL[2,4,3], H. POTTER[2,4,3], N. VERGARA[1,3,2,4];
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Abstract: Purpose and Rationale: Alzheimer's Disease (AD) is a neurodegenerative disorder that affects over 44 million individuals worldwide and is characterized by progressive cognitive impairment and irreversible damage to the central nervous system. Furthermore, AD can affect visual function, leading to reduced contrast sensitivity, depth perception, and visual field defects, among others. Notably, key indicators of AD histopathology within the brain are also present in the retina, including the deposition of amyloid-β (Aβ)-containing neuritic plaques, and neurofibrillary tangles (NFT) composed of hyperphosphorylated tau (pTau). Unfortunately, current models of AD are limited in their ability to recapitulate human retinal pathophysiology. Thus, the goal of this study was to develop the first human induced pluripotent stem cell (hiPSC)-derived organoid models of retinal AD histopathology that can be applied to the validation of potential treatments for AD retinopathy.
Methods: hiPSC lines from two healthy control (CTR) and two familial AD (FAD) male and female donors were used to generate retinal organoids (ROs) following the Zhong et al. 2014

protocol. The overall cellular composition and histopathological hallmarks of AD were assessed via Western blotting and immunofluorescence staining at 100 and 180 days of differentiation. Quantification was performed using Fiji for 2D images and three-dimensional automated reporter quantification (3D-ARQ) for whole-mount organoids. The sample size was at least 5 biological replicates per condition per time point. A t-test was used for pairwise comparisons and a p-value of 0.05 was considered statistically significant.
Results: The cellular composition and structure AD-ROs are similar to those of CTR-ROs. However, Aß plaque deposits and pathological pTau levels increased significantly in AD-ROs compared to CTR-ROs. Moreover, we developed a quantitative, fluorescence-based assay for Aß plaque detection amenable to translational research applications.
Conclusions: The AD-RO models developed and validated in this work recapitulate some of the key histopathological features of the human AD retina. These organoids constitute valuable tools for the screening and validation of candidate molecules with therapeutic potential.

Disclosures: E. James: None. **A. Vielle:** None. **H. Li:** None. **N.R. Johnson:** None. **H.J. Chial:** None. **H. Potter:** None. **N. Vergara:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.08

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01NS115977
Lisa Dean Moseley Foundation Grant

Title: Development of a next generation human induced pluripotent stem cell derived CNS model for tauopathy

Authors: *S. KOFMAN, X. SUN, L. IBRIC, L. QIANG;
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Abstract: The emergence of human induced pluripotent stem cell (hiPSC) technology has led to the development of several 3D *in vitro* modeling platforms for studies of neurodegenerative disorders. However, most of these models fail to capture the full range of implicated CNS cell types, including microvascular endothelial cells and microglia, thus limiting their utility in unveiling certain aspects of the disease. We sought to design and develop a 3D human assembloid model that incorporates populations of microvascular endothelial cells and microglia. This was done by combining hiPSC organoid-derived neuroectodermal cell components, human umbilical vein endothelial cells (HUVECs) modified by ETV-2, a transcription factor that promotes the plasticity endothelial cells, and hiPSC-derived microglia in an AggreWell system. Markers specific to neural progenitor cells, neurons, astrocytes, endothelial cells, microglia, and oligodendrocytes were confirmed within this assembloid model, as was the emergence of

physiologically relevant sub-structures such as the neuroepithelium and neurovascular units. Strikingly, more robust generation of mature astrocytes was obtained in assembloid cultures, compared to organoids that lacked additional endothelial cells. Moreover, consistent clustering of astrocytes and HUVECs was identified in the assembloids. These results suggest that inclusion of endothelium in the assembloid promotes not only astrocytic differentiation, but putative astrocyte-endothelium based neurovascular units. In order to validate the novel assembloid cultures in modeling neurodegenerative disorders, we applied them to explore cellular and molecular deficits in primary tauopathies. Patient derived hiPSCs harboring tauP301S mutation (seen in FTDP-17) and their isogenic controls were used to generate comparable assembloids. Given that both astrocytes and microglia have been implicated in several aspects of tauopathy, including enhanced neuroinflammation, we decided to examine morphological changes within these cell types and their association with pathological tau species. Through immunolabeling analyses, tauP301S assembloids were found to have increased levels of total tau and phosphorylated tau. Additionally, they contained more inflammatory rod-like microglia, and appeared to show emergence of hyperphosphorylated tau in astrocytes, microglia, and endothelial cells. Cytokine analysis revealed a significant increase in the proinflammatory profile in the tauP301S assembloids. Collectively, these results suggest our standardized assembloid cultures as an innovative model for studying tauopathy.

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Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.09

Topic: A.03. Stem Cells and Reprogramming

Support: Lieber Institute for Brain Development
The MGH Collaborative Center for X-Linked Dystonia-Parkinsonism
Maryland Stem Cell Research Fund

Title: Identifying the molecular drivers of somatic instability and neurodegeneration in the 3D organoid model of X-linked Dystonia-Parkinsonism

Authors: *L. D'IGNAZIO[1,3], B. ARAUJO[1], B. QAMAR[1], A. BARBOSA[1], A. S. FELTRIN[1], T. A. EVANS[1,3], A. LORENZETTI[1], T. SAWADA[1], A. GUERRERO ZUNIGA[1], Y. WANG[1], A. E. MCCORD[1], B. J. SHEEHAN[2], M. ZABOLOCKI[6], W. T. HENDRIKS[7,8], C. BARDY[6], D. C. BRAGG[7,8], A. C. M. PAQUOLA[1,3], J. A. ERWIN[1,3,4,5];
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Abstract: X-linked Dystonia-Parkinsonism (XDP) is an inherited adult-onset movement disorder affecting predominantly male of Filipino descent. Histopathological analyses unveiled a progressive differential degeneration in the adult neostriatum of XDP patients. At molecular level, a disease-specific SINE-VNTR-Alu (SVA) retrotransposon insertion occurs within intron 32 of *TAF1* gene, and the length of the hexamer repeat within the pathogenic SVA retrotransposon is inversely correlated with age of disease onset. Current efforts focus on rescuing altered *TAF1* splicing, however how SVA-driven pathological mechanisms contribute to XDP is yet unknown. Here, in order to recapitulate the mature and complex XDP cellular system, we drove the differentiation of female carrier and male control, XDP, and isogenic SVA-deleted XDP induced pluripotent stem cells (iPSCs) into ventral forebrain organoids. Through extensive phenotypic, functional, and transcriptomic analyses, we found that ventral forebrain organoids from XDP patients demonstrate decreased calretinin and full-length *TAF1* expression, somatic DNA expansion of the SVA hexamer repeat during organoid maturation, apoptosis, and decreased electrical activity. Serving as a valuable tool for disease modeling, XDP ventral forebrain organoids recapitulated key molecular features of the disease, and uncovered novel insights of XDP etiology.

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Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.10

Title: WITHDRAWN

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.11

Topic: A.03. Stem Cells and Reprogramming

Support: A Cure for Ellie

Title: Enhancing dars2 expression via adeno-associated virus 9 as a potential therapeutic approach for leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation

Authors: *A. RATAJCZAK[1], Y. LIANG[2], M. JANOWSKI[2], P. WALCZAK[2], A. FATEMI[1], C. NEMETH[1];
[1]Moser Ctr. for Leukodystrophies, Kennedy Krieger Inst., Baltimore, MD; [2]Dept. of Diagnos. Radiology and Nuclear Med., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Leukoencephalopathy with Brainstem and Spinal Cord Involvement and Lactate Elevation (LBSL) is a rare, heritable, and progressive neurological disease primarily affecting motor function in its clinical presentations. LBSL is caused by mutations in the *DARS2* gene, which encodes for mitochondrial Aspartyl-tRNA Synthetase - a protein that charges aspartic acid to its cognate tRNAs in the mitochondria. Most patients present as compound heterozygotes, with one allele expressing a null mutation and the other exhibiting a splice-site mutation between intron 2 and exon 3, forming a partially functional allele and allowing for production of some protein albeit at lower levels than normally expected. Therefore, increasing the amount of functional DARS2 protein is a major therapeutic goal in the treatment of LBSL. Adeno-Associated Virus (AAV) vectors have become common agents of gene therapy in recent years. This is due to their ability to transduce human cells, low toxicity, and persistence over time without genomic integration in non-dividing cells - making them particularly useful in treatments of neurological diseases. Many serotypes exist, but AAV9 has shown a high affinity for neuronal cells. Thus, in this study we developed an AAV9 vector containing a DARS2 plasmid for transduction of LBSL patient cells. Prior to transduction, LBSL motor neurons were derived from patient iPSCs according to an established protocol, and these were allowed to mature to Day 16 *in vitro*. Transduction was performed with a multiplicity of infection of approximately 30,000 viral genomes/cell. Media was changed after overnight exposure, and the expression of three different exons - 3,6, and 17 - was analyzed in triplicate 10 days post-treatment via RT-qPCR relative to normalized values in an untreated control group of LSBL cells. All exons exhibited increased expression, with the following average increases observed: exon 3 - 1821.775 ± 105.402, exon 6 - 164.782 ± 2.638, and exon 17 - 158.423 ± 6.806-fold higher expression. These results suggest efficacy of the AAV9 vector in enhancing *DARS2* expression *in vitro.* However, further work is needed to verify these results, and an *in vivo* model will be necessary before advancing toward clinical investigation.

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Poster

600. Advances in Disease Modelling With Stem Cells

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Program #/Poster #: 600.12

Topic: A.03. Stem Cells and Reprogramming

Support: DOD Grant W81XWH1910229
JHU COVID-19 Bridge Grant 80056510

Title: Axonal mRNA Profiling from iPSC-derived Spinal Motor Neurons with ALS-linked Mutations

Authors: *M. JAMES, A. VENKATESAN, M. H. FARAH;
Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Local mRNA translation and protein production occur within axons, and axons have been shown to contain their own subsets of diverse RNA transcripts transported from the cell bodies. The regulation of axonal RNA has been implicated in Amyotrophic Lateral Sclerosis (ALS) pathogenesis. ALS, a devastating neurodegenerative disease, is the most common motor neuron disease in adults, with minimal treatment options and a short patient survival time beyond symptom onset. Early disease pathogenesis includes denervation at the neuromuscular junction (NMJ), dying-back axonopathy, and distal axon degeneration preceding cell death. RNASeq studies have been done looking for differences in gene expression profiles between iPSC-derived motor neurons from patients with ALS disease-causing mutations and controls, as these may give key insights into disease mechanisms. However, given early disease pathology, axonal gene expression specifically may contribute to disease. As such, we've focused this work on isolating axonal RNA. We have cultured induced pluripotent stem cell (iPSC)-derived motor neurons in microfluidic devices that allow us to isolate the distal axons from the cell bodies. By using modified microfluidic devices with chambers separated by two sets of channels in a row rather than one, we have been able to completely sequester cell bodies away from axons to isolate pure populations of axons without contamination from cell bodies, as single-cell RNAseq has shown that a single neuron can have more RNA that pooled axons. We have also been able to extract high quality RNA from control and ALS-mutant cells for sequencing from the spatially separated compartments, allowing us to analyze axonal RNA expression profiles independent of neuronal cell body expression. Presently, we are conducting bioinformatic analysis to interrogate axonal mRNA profiles in the context of ALS-disease causing mutations.

Disclosures: M. James: None. **A. Venkatesan:** None. **M.H. Farah:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.13

Topic: A.03. Stem Cells and Reprogramming

Support: Millken

Title: Atp synthase c-subunit plays an important role in bipolar disease

Authors: *L. SHEN[1], E. A. JONAS[2];
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Abstract: AbstractLei Shen and Liz Jonas, 2022
Stem cell-derived 3D human brain organoids have the potential to recapitulate features of the human brain with greater complexity than 2D models and are increasingly being applied to model diseases affecting the central nervous system. Neurons derived from patient (hiPS) cells have also been used to model highly heritable yet idiopathic psychiatric disorders. Studies of hippocampal dentate gyrus-like neurons derived from patients with bipolar disorder have previously revealed mitochondrial abnormalities and neuronal hyperexcitability compared with healthy controls. A previous report from our lab had shown that neurons from an autism model mouse and patient cells had a leak in the ATP synthase caused by an imbalance of ATP synthase components. The membrane bound portion of the ATP synthase (c-subunit) was overexpressed compared to the assembled ATP synthase and formed a leaky channel. We therefore determined if ATP synthase stoichiometry was affected in bipolar disorder in patient derived neurons. We measured ATP synthase subunits' protein expression in one bipolar patient and one control monocyte-derived pluripotent stem cells (iPSC) and in human medial ganglionic eminence-like organoids (hMGE) and cortical-like organoids (hCO) from the bipolar patient and the control. We found ATP synthase β-subunit was decreased but c-subunit was increased in both types of organoids at 75 days compared to those of the healthy control, whereas in the iPSCs, we found enhanced c-subunit expression and reduced total VDAC levels. We hypothesize that the normal aerobic glycolytic metabolism of developing neurons is persistent in bipolar patients' neurons' due to hyperexcitability of ATP synthase c-subunit leak channel (ACLC), leading to a persistent glycolytic metabolic phenotype at later developmental stages with high lactate production and reversal of ATP synthase to the hydrolytic mode. We will next determine the location of ATP production (mitochondria vs. glycolysis) in these two types of organoids to further study the metabolism. To determine hyperexcitability of ACLC, we will record ATP synthase c-subunit leak channel activity in the BP and healthy control human organoids.

Disclosures: L. Shen: None. **E.A. Jonas:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

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Program #/Poster #: 600.14

Topic: A.03. Stem Cells and Reprogramming

Support: DOD Grant N00014-21-S-F002
DOD Grant S-10780-01
DOD Grant S-10743-02

Title: Using brain organoids to model neural network dynamics during traumatic brain injury

Authors: M. J. SILVOSA[1], N. ROMO MERCADO[1], N. MERLOCK[3], S. VIDHATE[4], R. MEJIA-ALVAREZ[5], T. T. JUAN[6], A. M. WILLIS[6], ***Z. R. LYBRAND**[2];
[1]Biol., [2]Texas Woman's Univ., Denton, TX; [3]UT San Antonio, San Antonio, TX; [4]Dept. of Radiology and Imaging, NIH Clin. Ctr., Bethesda, TX; [5]Michigan State Univ., Lansing, MI; [6]59th Med. Wing United States Air Force, Lackland AFB, TX

Abstract: Traumatic brain injury (TBI) is an accumulation of mechanical stressors including pressure, shear, tension, and cavitation on brain tissue that can cause cellular death, acute physiological cellular changes, and chronic neuropathology that are associated with neurodegenerative diseases like Alzheimer's disease and Chronic Traumatic Encephalopathy (CTE). However, due to the complexity of the tissue stress, it is unclear the role of each mechanical parameter in changing neurological function. In this study, we use a benchtop blast device to isolate pressure across frequency and amplitude to characterize the change in complex neural network dynamics. To accomplish this, we grew pluripotent stem cells into brain organoids and exposed them to lower (500Hz), mid (3000Hz), and higher (5000Hz) frequency pressure waves at multiple amplitudes (250kPa and 350kPa). The brain organoids develop a putative cortical niche with astrocytes and neurons that replicate the architecture and physiology of human neural networks. After exposure, organoids were immediately plated onto a multielectrode array (MEA) and network activity was recorded 1 hour and 24 hour after blast. We observed an amplitude dependent response to neural activity and network oscillations. With lower amplitude pressure waves (250kPa), there was a frequency dependent response in the suppression of individual local field potential (LFP) activity, enhancement of population spike activity, and inhibition of network oscillation between 1-10Hz. Furthermore, these network oscillations, in response to highest frequency pressure waves, become desynchronized between neighboring electrodes. Additionally, these "mild" parameters resulted in limited apoptosis measured by activated caspase 3. At high amplitude pressure waves (350kPa), the same frequency of waveforms resulted in drastically different neurophysiological responses ultimately leading to exaggerated network dynamics and pervasive cellular death. This suggests that the amplitude of pressure waves may be more detrimental than frequency. Furthermore, we have defined thresholds to pressure waves that can disrupt neural network activity.

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Poster

600. Advances in Disease Modelling With Stem Cells

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Program #/Poster #: 600.15

Topic: A.03. Stem Cells and Reprogramming

Support: NIH/NIAID R01 AI132414-01A1

Title: Understanding Cell Type Specific Impacts of HCMV Infection and Viral Entry within 3D Cortical Organoids and 2D Neural Progenitor Cells

Authors: *B. O'BRIEN[1], M. SCHUMACHER[2], S. TERHUNE[2], A. EBERT[1], R. MOKRY[3]; [1]Cell Biology, Neurobiology, and Anat., [2]Microbiology and Immunol., Med. Col. of Wisconsin, Milwaukee, WI; [3]Arizona Univ., Tucson, AZ

Abstract: Human cytomegalovirus (HCMV) is a beta herpesvirus that can cause severe congenital birth defects including microcephaly, vision loss, and hearing loss. In our previous work, we infected human induced pluripotent stem cell derived cortical organoids using HCMV TB40/E expressing eGFP and demonstrated that HCMV infection significantly downregulates genes involved in several critical neurodevelopmental pathways. However, what remains to be elucidated is how HCMV enters neural tissues to cause these transcriptional effects. HCMV is known to use the trimeric complex (TC) to infect fibroblasts, whereas the pentameric complex (PC) is thought to be needed along with the TC to infect epithelial cells. Studies in the literature also suggest that the virus adopts the complex associated with the cell type used for propagation leading to higher levels of a given complex on its particle. As neural tissues are epithelial derived, we hypothesize that epithelial propagated virus will generate a more robust infection than fibroblast propagated virus in neural tissues due to increased expression of the PC. We observe significant increases in the number of viral genomes present and amount of viral spread and penetrance using epithelial propagated TB40/E virus versus fibroblast propagated virus when infecting neural tissues. Further, these tissues develop multinucleated structures within 72 hours of infection in epithelial propagated virus conditions only. Using flow cytometry and immunofluorescence, we found that the TC interactors PDGFRa and TGFßRIII and PC interactor Nrp2 are the most abundantly expressed in organoids and neural progenitor cells (NPCs). However, in neutralizing antibody studies, we found that pre-treatment with antibodies against viral glycoprotein gB is the most successful at reducing viral genomes and entry in NPCs compared with antibodies blocking individual receptors. We also find that pre-treatment with gB antibody rescues expression of previously identified neurodevelopmental gene targets. Overall, these data suggest that epithelial propagated TB40/E virus is more efficient at entering neural tissues, but it may not be reliant on PC components. It also presents evidence that pretreatment with viral glycoprotein gB antibody can limit downstream neurodevelopmental impacts of infection.

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Poster

600. Advances in Disease Modelling With Stem Cells

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Program #/Poster #: 600.16

Topic: A.03. Stem Cells and Reprogramming

Support: BBRF 28771
CIRM EDUC4-12804

Title: A Human 3D neural assembloid model for SARS-CoV-2 infection

Authors: *L. WANG[1], J. G. GLEESON[2];
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Abstract: Clinical evidence suggests the central nervous system (CNS) is frequently impacted by SARS-CoV-2 infection, either directly or indirectly, although mechanisms remain unclear. Here we integrate human pericyte-like cells into cortical organoids to generate pericyte-containing cortical organoids (PCCOs). The presence of pericyte-like cells elicited astrocytic maturation and production of basement membrane components; features attributed to a neurovascular unit-like structure. Unlike traditional cortical organoids, PCCOs demonstrated robust SARS-CoV-2 infection, with pericyte-like cells serving as viral 'replication hubs', inducing infection of astrocytes, and mediating inflammatory type I interferon transcriptional responses. Therefore, PCCOs support SARS-CoV-2 entry and replication in neural tissue, and PCCOs serve as an experimental model for neural infection.

Disclosures: L. Wang: None. **J.G. Gleeson:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 600.17

Topic: A.03. Stem Cells and Reprogramming

Support: NINDS grants R21NS114775 and R01NS114245 to JS

Title: Patient-ipsc-derived astrocytes to model neuroinflammatory response in x-linked adrenoleukodystrophy

Authors: *P. PARASAR[1], N. KAUR[1], L. POISSON[2], J. SINGH[1];
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Abstract: Background. X-linked adrenoleukodystrophy (X-ALD) is ABCD1-mutation-driven peroxisomal disorder and is characterized by accumulation of very long chain saturated fatty acids in brain, nervous system, and adrenal gland. Two forms of X-ALD are fatal cerebral inflammatory and demyelinating ALD form (cALD) and milder adrenomyeloneuropathy (AMN). **Problem:** Current mouse models are unable to mimic the severe cALD phenotypic in X-ALD. Furthermore, inaccessibility to patient-tissue samples presents a caveat in designing a directly translatable cellular model to study the disease mechanism and effective drug discovery. **Objective.** In this study, we aim to differentiate astrocytes from patient-fibroblast-derived

induced pluripotent stem cell (iPSCs) and to investigate molecular and etiopathogenetic mechanisms of cALD and AMN. **Methods.** We reprogrammed fibroblasts from control, adrenomyeloneuropathy (AMN), and cerebral adrenoleukodystrophy (cALD) patients carrying ABCD1 mutation to generate iPSCs. iPSCs were differentiated into astrocytes and used to perform transmission electron microscopy, gene expression, Western blotting, enzyme-linked immunosorbent assay, and lipidomics to characterize phenotypic and molecular features of patient-derived AMN and cALD astrocytes. **Results.** We confirmed the deletion of ABCD1 gene and identified ABCD-1 mutation-driven very long chain fatty acid (VLCFA) accumulation in AMN ($p < 0.01$) and cALD astrocytes ($p < 0.001$) compared to control. cALD astrocytes showed increased glycolysis, increased signal transducer and transcription activator (STAT) 3 activation ($p < 0.001$), and reduced expression of anti-inflammatory cytokines such as arginase-1 ($p < 0.05$) and mannose receptor C-type-1 ($p < 0.05$). We conclude that toll-like receptor-signaling via MyD88 and NF-κB (p52 and p65) induced STAT3 is potential contributor to inflammatory milieu in cALD while interleukin-6 classical-induced anti-inflammatory cytokine production and increased chemotactic CCL2
(MCP-1) production in AMN potentially favors microglial recruitment protecting its further progression. **Interpretation**: We demonstrate for the first time that patient iPSC-derived astrocytes mimic and recapitulate neuroinflammatory and biochemical defects of X-ALD and provide an human *in vitro* cellular system to study X-ALD.

Disclosures: P. Parasar: None. **N. Kaur:** None. **L. Poisson:** None. **J. Singh:** None.

Poster

600. Advances in Disease Modelling With Stem Cells

Location: SDCC Halls B-H

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Topic: A.03. Stem Cells and Reprogramming

Support: Academy of Finland (SH 330707; 335937, SN 336665)
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The Finnish Cultural Foundation (SH)
The Doctoral Programme in Medicine, Biosciences and Biomedical Engineering, Tampere University (JL)

Title: Human iPSC-derived microglia for in vitro modeling of multiple sclerosis

Authors: *J. LOTILA[1], T. HYVÄRINEN[1], H. JÄNTTI[4], S. OHTONEN[4], H. SKOTTMAN[2], L. AIRAS[5], T. MALM[4], S. NARKILAHTI[3], S. HAGMAN[1];
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of Eastern Finland, Kuopio, Finland; [5]Clin. Neurosciences, Univ. of Turku and Neurocenter, Turku Univ. Hosp., Turku, Finland

Abstract: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) which causes demyelination, neuroinflammation, astrogliosis and axonal injury. Understanding of pathophysiological mechanisms of MS is still incomplete, but overactivation of microglia have been associated to pathogenesis of MS, and especially to the disease progression. Microglia are the primary immune cells of the CNS which have several homeostatic and inflammatory functions.
The aim of this study was to produce MS patient-specific microglia-like cells (iMGs) from human induced pluripotent stem cell (hiPSC) lines and explore their homeostatic and inflammatory dysfunctions relevant for MS pathogenesis. We generated six MS patient-specific hiPSC lines from peripheral blood mononuclear cells utilizing genome integration-free Sendai virus reprogramming. hiPSC lines were characterized for pluripotency markers and tri-lineage differentiation potential. Healthy control and MS patient-derived hiPSCs were differentiated into iMGs, and their homeostatic functions and inflammatory responses were investigated upon stimulation with lipopolysaccharide (LPS) and interferon gamma (IFN-γ). iMGs were characterized by staining for Iba1, TMEM119 and P2RY12, by analyzing their phagocytosis capacity with pHrodo Green Zymosan Bioparticles, and by studying their calcium signaling and secretion of inflammatory molecules. NF-κB signaling was investigated with NF-κB p65 staining and western blot analysis of NF-κB p65, pNF-κB and IκBα.
Our results showed that produced hiPSC lines expressed pluripotency markers, differentiated into three germ layers and had a normal karyotype. All lines differentiated into iMGs expressed microglial markers, and were functionally displaying phagocytic capacity and calcium transients. Importantly, iMGs responded to inflammatory stimulations by morphological change from ramified to ameboid morphology and induced their phagocytosis capacity. Furthermore, inflammatory stimulation induced activation of NF-κB signaling and increased cytokine secretion. In summary, MS-hiPSC lines were produced successfully and differentiated iMGs were functional. These results suggest that hiPSC-derived iMGs are valuable research tools for human stem cell-based disease modeling to obtain further in-depth knowledge about the mechanisms underlying MS and novel drug targets.

Disclosures: **J. Lotila:** None. **T. Hyvärinen:** None. **H. Jäntti:** None. **S. Ohtonen:** None. **H. Skottman:** None. **L. Airas:** None. **T. Malm:** None. **S. Narkilahti:** None. **S. Hagman:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.01

Topic: A.03. Stem Cells and Reprogramming

Title: A human multi-organoids-based platform to model neurodevelopmental disorders following physiological stimulation

Authors: *J.-P. URENDA[1], V. TRUONG[1], J. EICHENBAUM[1], A. ATAMIAN[1], A. DEL DOSSO[1], C. LOIS[2], M. MCCAIN[1], G. QUADRATO[1];
[1]USC, Los Angeles, CA; [2]Caltech, Pasadena, CA

Abstract: The advent of 3D human pluripotent stem cell-derived brain organoids has allowed for the monitoring and assessment of several early developmental processes, including progenitor biology, emergence of cellular diversity, and neuronal activity. However, current brain organoids are not suitable for modeling later stages of brain development as they do not recapitulate many biological features necessary to interrogate complex neurodevelopmental (ND) phenotypes due to a lack of stereotypic macroscale anatomy and limited activity-dependent maturation. To address these issues, we aim to pioneer a multi-organoid-on-chip platform that recapitulates the functional connectivity of the human visual system, which will facilitate the establishment of discrete long-range functional connections between organoids and allow for physiological light-based stimulation of neurons. We have successfully cultured 5 weeks-old cortical organoids within a multichambered microfluidic device for 2 months. Importantly, while grown in static conditions, these cortical organoids can maintain cell viability and differentiation potential, as shown by expression of $CTIP2^+$ and $SATB2^+$ cortical neurons that display functional activity. Separately, we have induced neurite extension and directed axonal growth cone guidance through microchannels using collagen-based hydrogels containing known chemoattractants. We will integrate retinal, thalamic, and cortical organoids into our microfluidic device with hydrogel coated microchannels to functionally connect organoids. We will validate functional connectivity and characterize the ND impact of light-based stimulation of retinal organoids on cortical neurons using single cell RNA-seq and electrophysiological recordings. This project will elucidate the role of activity-dependent neuronal stimulation in shaping macro-circuit connectivity during human brain development with single cell resolution allowing for future interrogation of ND disorder pathologies.

Disclosures: J. Urenda: None. **V. Truong:** None. **J. Eichenbaum:** None. **A. Atamian:** None. **A. Del Dosso:** None. **C. Lois:** None. **M. McCain:** None. **G. Quadrato:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.02

Topic: A.03. Stem Cells and Reprogramming

Title: Generation of functional cerebellar organoid from human pluripotent stem cells

Authors: *A. ATAMIAN, M. BIRTELE, T. NGUYEN, A. DEL DOSSO, A. SETH, K. HITON, G. QUADRATO;
USC, LOS ANGELES, CA

Abstract: The development of the human cerebellum is quite more complex and protracted than most mammals leading to an increase in susceptibility to neurodevelopmental disease. The evolutionary expansion of the cerebellum began in parallel lineages of apes, but only rapidly increased in the great ape clade, suggesting a role in acquiring human-specific traits. Human pluripotent stem cell-derived brain organoids have emerged as an effective in vitro system to interrogate the development and disease of multiple brain regions; however, a protocol that models the developing human cerebellum with high fidelity is not yet available. Here we report the establishment of a robust protocol that can reproducibly generate the cellular diversity of the human cerebellum within and across multiple cell lines. The human cerebellar organoids exhibit an organized laminar layering with spatially segregated ventricular and rhombic lip progenitor zones that give rise to functional, inhibitory and excitatory cerebellar neuronal subtypes respectively. In addition, extending the time in culture increased the level of maturation of these organoids, which were identified through changes in transcriptomic profiles, increased neurite outgrowth, and increased coordinated network activity. This demonstrates that cerebellar organoids are suitable to model various aspects of human cerebellar development and disease, including disruptions in developmental trajectories and cerebellar circuit functionality.

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Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.03

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS096282, NS076352, NS086604, U54 HD090256

Title: Activin a specifies locus coeruleus norepinephrine neurons from human pluripotent stem cells

Authors: *Y. TAO, X. LI, Q. DONG, L. KONG, A. PETERSEN, Y. YAN, K. XU, S. ZIMA, Y. LI, M. AYALA, S. MATHIVANAN, Q. CHANG, S.-C. ZHANG;
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Abstract: Central norepinephrine (NE) neurons, mainly located in the Locus coeruleus (LC), play roles in a wide range of behavioral and physiological processes. How the human LC-NE neurons develop and what roles they play in the pathophysiology of human diseases is poorly understood, partly due to the unavailability of functional human LC-NE neurons. Here we established a method for the efficient generation of LC-NE neurons from human pluripotent stem cells by identifying a novel role of ACTIVIN A in regulating the LC-NE transcription factors in the dorsal rhombomere 1 (r1) progenitors. The in vitro generated human LC-NE neurons not only display extensive axonal arborization and release/uptake NE, but also exhibit the pacemaker

activity, calcium oscillation, and in particular chemoreceptor activity in response to CO_2. The LC-NE neurons engineered with a NE sensor reliably reported the extracellular NE level. The availability of functional human LC-NE neurons enables investigation of their roles in the pathogenesis of and development of therapeutics for neural psychiatric and degenerative diseases.

Disclosures: **Y. Tao:** None. **X. Li:** None. **Q. Dong:** None. **L. Kong:** None. **A. Petersen:** None. **Y. Yan:** None. **K. Xu:** None. **S. Zima:** None. **Y. Li:** None. **M. Ayala:** None. **S. Mathivanan:** None. **Q. Chang:** None. **S. Zhang:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

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Program #/Poster #: 601.04

Topic: A.03. Stem Cells and Reprogramming

Support: MRC Grant UKDRI-3005
UKDRI Grant DRI-TRA2021-02

Title: Lineage tracing of LMX1A$^+$ derivatives in ventral midbrain astrocyte differentiation identifies distinct astrocyte populations with ventral midbrain or alternative identity

Authors: ***Z. LI**[1], L. F. CARDO[1], M. ROKICKI[1], C. WEBBER[1], M. LI[2,3];
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Abstract: Recent evidence has pointed to a role of astrocytes in the etiology of Parkinson's disease (PD). Meanwhile, astrocyte heterogeneity across brain regions has also gained a better appreciation, with both transcriptomic and functional differences reported in both animal and stem cell-derived models. Since PD specifically affects midbrain dopaminergic (mDA) neurons in substantia nigra, several studies have attempted to generate ventral midbrain astrocytes from the stem cell-derived mDA lineage to model PD *in vitro*. However, in the absence of lineage tracing or *in vivo* reference, the lineage identity of these astrocytes was poorly supported. To tackle these challenges, we utilized an LMX1A-BFP reporter system in a KOLF2 induced pluripotent stem cell line background to purify and faithfully lineage trace LMX1A$^+$ mDA lineage progenitors during astrocyte differentiation. This reporter cell line can constitutively express blue fluorescent proteins (BFP) upon the expression of LMX1A, which exclusively encompasses the dopaminergic domain in the developing ventral midbrain. Using this cell line, we generated highly enriched mDA lineage progenitors expressing LMX1A, FOXA2 and OTX2, of which 85.48±0.76% (SEM; n=4) were BFP$^+$ by flow cytometry. The BFP$^+$ population was purified using fluorescence-activated cell sorting, and both the sorted and unsorted population were differentiated towards astrocytes. Progenitor expansion and astrocytic induction of the unsorted progenitors resulted in a loss of BFP$^+$ progenitors in the unsorted culture but not in the

purified BFP^+ culture. The remaining BFP^- cells in the unsorted culture did not express OTX2, suggesting a lack of midbrain identity, whereas the purified BFP^+ population remained mostly $OTX2^+$. Both the remaining BFP^- progenitors in the unsorted culture and the sorted BFP^+ progenitors can be differentiated into astrocytes. Astrocytes from both lineages expressed ALDH1A1, AQP4, CD44, CD49f, EAAT2, NFIA, S100B, and SOX9, but not GFAP. Despite similar marker expression, astrocytes of the two lineages displayed different functional properties and maturity. Using SMARTseq single cell RNA sequencing, we characterised distinct molecular profiles of astrocytes derived from the unsorted and the purified BFP^+ population. The transcriptomic profile of BFP^+ astrocytes resembled that of the glial population in published human fetal midbrain datasets, supporting the midbrain identity of these BFP^+ astrocytes. Our study highlights the need for better and more careful characterisation of the regional identity of stem cell-derived astrocytes.

Disclosures: Z. Li: None. **L.F. Cardo:** None. **M. Rokicki:** None. **C. Webber:** None. **M. Li:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

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Program #/Poster #: 601.05

Topic: A.03. Stem Cells and Reprogramming

Support: CONACYT A1-S-8450
INPER 2019-1-40

Title: The amniotic epithelium confers a bias to differentiate toward the neuroectoderm lineage in human embryonic stem cells

Authors: *D. AVILA-GONZÁLEZ[1,2], C. BARRAGÁN-ÁLVAREZ[1], W. PORTILLO[3], A. MOLINA[2], E. DÍAZ-MARTÍNEZ[1], N. E. DIAZ[2];
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Abstract: Human embryonic stem cells (hESC) derive from the epiblast and can differentiate into all the lineages that constitute an organism. On the other hand, the neuroectoderm is specified in the human epiblast once morphogenesis begins. Previously, our group demonstrated that hESC could be derived and maintained on human amniotic epithelial cells (hAEC). Here, we evaluated whether the hAEC-hESC co-culture conferred a differentiation potential different from standard conditions (inactivated mouse embryonic fibroblasts, iMEF). Our data demonstrate that hESC-hAEC interaction showed a gene downregulation expression associated with the endoderm and mesoderm and an increase in the ectoderm lineage genes, specifically from the anterior neuroectoderm (FEZ1, LHX5, SIX3, OTX1/2) as compared with the hESC-iMEF condition.

When hESC were cultured under feeder layer-free conditions with an hAEC-conditioned medium, the expression of anterior neuroectoderm-related genes decreased, suggesting that cellular interaction between hESCs and the amniotic epithelium is required to gain this molecular state differently from conventional primed hESC. Next, we challenged it to differentiate towards the neural lineage, hESC-hAEC showed an increase in mature (MAP2+) and cortical (FOXP2+, GAD67+, CTIP2+) neurons number as compared with hESC-iMEF. To elucidate the possible mechanism(s), we analyzed the phosphorylated kinase proteomes, which showed an SRC kinase, STAT3, ERK and AKT signaling pathways upregulation. Thus, each pathway was inhibited by a specific small molecule and the expression of anterior neuroectoderm-related genes, as well as the potential for neural differentiation, were evaluated. PI3K/AKT pathway inhibition did not show any effect. However, SRC, ERK and STAT3 single inhibition altered the neural differentiation potential of hESC-hAEC condition demonstrated through a decrease in neural progenitors and mature neurons. In contrast, there were no alterations in hESC-iMEF condition when inhibiting the SRC and ERK pathways. Our results suggest that hAEC-hESC interaction promotes a biased potential to neuroectoderm mediating by SRC and ERK signaling.

Disclosures: **D. Avila-González:** None. **C. Barragán-Álvarez:** None. **W. Portillo:** None. **A. Molina:** None. **E. Díaz-Martínez:** None. **N.E. Diaz:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.06

Topic: A.03. Stem Cells and Reprogramming

Title: Dendritic spine development and functional maturation in human iPSC-derived neurons

Authors: *W. LIN[1], S. SHIOMOTO[1], S. YAMADA[1], H. WATANABE[1], Y. KAWASHIMA[1], Y. SEKINO[2,3];
[1]Ricoh Company, Ltd., Kawasaki, Japan; [2]The Univ. of Tokyo, Tokyo, Japan; [3]Inst. for Drug Discovery Innovation, Tokyo, Japan

Abstract: Advances in using induced pluripotent stem cell (iPSC)-derived neurons hold great promise for disease modeling and regenerative medicine. However, synaptic maturation has often been reported to be limited in iPSC-derived neurons cultured *in vitro*, which makes functional assays challenging. The overexpression of specific transcription factors (TF) in iPSCs allows a rapid and efficient generation of differentiated neurons and is thus expected to facilitate the evaluation of further maturation stages. In this study, we characterized the transcriptional and morphological changes associated with the late-stage development of dendritic spines and excitatory synapses in TF-induced iPSC neurons. We performed a comprehensive time-course evaluation of neuronal marker expression and electrophysiological properties in long-term cultures. RNA-sequencing (RNA-seq) showed that neuronal markers were differentially expressed and that synapse-related genes were progressively upregulated during a long-term

maturation phase following the initial neurogenesis phase. Immunocytochemistry confirmed the recruitment of major pre-and postsynaptic molecules along maturing dendrites. Notably, the subcellular distribution of drebrin, an actin-binding protein involved in the morphology and dynamics of dendritic spines, was shown to change over time as its embryonic isoform switched to the mature brain-specific isoform. Finally, synaptic functionality was evaluated by visualizing the effects of glutamate stimulation on drebrin cluster density and measuring neuronal network activity on microelectrode arrays (MEA). Dynamic cellular responses suggested postsynaptic NMDA-receptor dependent regulations similar to mechanisms previously demonstrated in rodent cortical cell cultures. Our results show for the first time that TF-induced iPSC neurons can robustly reach dendritic spine maturation, which would be essential for developing pharmacological applications relevant to human brain functions and disorders.

Disclosures: **W. Lin:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **S. Shiomoto:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **S. Yamada:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **H. Watanabe:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **Y. Kawashima:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **Y. Sekino:** 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.; Ricoh Company, Ltd..

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.07

Topic: A.03. Stem Cells and Reprogramming

Title: A Human Pluripotent Stem Cell-Derived Organoid Model for Recapitulation of Central Nervous System (CNS) Barrier and Fluid Secretion Functions of the Choroid Plexus

Authors: *L. H. CHEW[1], A. C. EAVES[1,2], S. A. LOUIS[1], E. KNOCK[1];
[1]STEMCELL Technologies, Vancouver, BC, Canada; [2]Terry Fox Lab., BC Cancer, Vancouver, BC, Canada

Abstract: The choroid plexus plays a critical role in forming the blood-cerebrospinal fluid barrier and is responsible for generating cerebrospinal fluid (CSF) in the central nervous system (CNS). Along with the blood-brain barrier, the blood-CSF barrier functions to restrict entry of harmful pathogens but also therapeutics into the CNS. Recently, a human pluripotent stem cell (hPSC)-derived three-dimensional organoid model of the choroid plexus was developed and proof-of-concept experiments demonstrated the potential of this technology for biomarker discovery and blood-CSF permeability assays (Pellegrini et al., Science 2020). Here we present data from our STEMdiff™ Choroid Plexus Organoid Differentiation Kit, which is based on the above publication, for generating these CNS barrier-forming organoids. Single-cell suspensions

of hPSCs were cultured in 96-well round-bottom plates in embryoid body formation medium for 5 days at 37°C. The aggregates were then switched to an induction medium for 2 days (days 5 - 7), then embedded in Corning® Matrigel® and grown in expansion medium for 3 days (days 7 - 10). The culture medium was then switched to a differentiation medium for 5 days (days 10 - 15). On day 15, medium was switched to a maturation medium. At ~day 30, choroid plexus organoids were observed to generate large cysts (> 70%, 112/144 organoids, n=6 hPSC lines) and exhibited upregulation of choroid plexus markers (TTR, CLIC6, and AQP-1) and downregulation of cortical markers (PAX6, MAP2, and FOXG1) compared to unpatterned cerebral organoids using RT-qPCR and immunostaining (n=6 hPSC lines, 3 organoids per cell line). Cystic fluid was extracted at ~day 50 for analysis of CSF proteins, and the barrier function was tested using low-molecular weight FITC-dextran. Both clusterin and IGF2, proteins found in high abundance in human CSF, were detected in the CSF-like fluid using Western blot. We further found that FITC-dextran was excluded from the cyst compartment of the choroid plexus organoids. Our results demonstrate that STEMdiff™ Choroid Plexus Organoid Kits can generate in vitro human models that recapitulate the CNS barrier and CSF-like secretion functions of the choroid plexus.

Disclosures: **L.H. Chew:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **A.C. Eaves:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); STEMCELL Technologies. **S.A. Louis:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **E. Knock:** A. Employment/Salary (full or part-time):; STEMCELL Technologies.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.08

Topic: A.03. Stem Cells and Reprogramming

Support: MOST 109-2314-B-281 -002 -MY3

Title: Downregulation of S100A16 expression facilitates process branching and extension in astrocyte differentiation through the translocation of CKAP4

Authors: *C.-C. CHIEN[1,3], Y.-C. CHENG[2,4], C.-J. HUANG[2,5], Y.-J. LEE[3];
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Abstract: We have previously demonstrated the knockdown of the heat-shock protein 27 (HSP27, also termed HSPβ1 or HSP25) and S100 calcium-binding protein A16 (S100A16) by specific short-hairpin RNA (shRNA) triggered placenta-derived multipotent cells (PDMCs) to differentiate into functional astrocytes without any chemical induction *(published in Stem Cell*

Rev Rep. 2022 Feb;18(2):839-852. doi: 10.1007/s12015-021-10319-3.). To further explore possible molecules and mechanisms involved in this process, we first applied co-immunoprecipitation, mass spectrometry analysis and immunoblotting to identify a protein cytoskeleton-associated protein 4 (CKAP4, also known as CLIMP63) interacted with S100A16 in the differentiated astrocytes. We further constructed lentivirus containing shS100A16 in combination with either overexpressed CKAP4_WT (wild type) or CKAP4_3E (phosphorylation mimic, with three serine positions replaced by glutamic acid near the N terminal) to test their possible cellular effects. After transfection, immunofluorescence and morphometric analysis demonstrated that there was no difference in dendritic development between CKAP4_WT overexpressed and controlled PDMCs. However, in the presence of shS100A16, CKAP4_WT overexpression induced more dendritic branches in co-transfected PDMCs. As for the comparison, CKAP4_3E overexpression alone increased dendritic branches numbers to about 2 folds and may even increase to 3 fold in the presence of shS100A16. The subcellular distribution of CKAP4 demonstrated by high magnification confocal microscopy is more intriguing. When PDMCs were induced into astrocytes, CKAP4 protein translocated from endoplasmic reticulum (ER) to membrane and extruded within astroglial main processes but not in the marginal small protruding. Considering CKAP4 protein was known to bind microtubules in ER and interacted with Microtubule-associated Protein-2, our results implies that binding of S100A16 with CKAP4 in PDMCs prevents CKAP4 from binding to microtubules in ER. While in the process of astrocyte differentiation, decreased S100A16 protein level releases CKAP4 for translocation to membrane and benefit the formation of astrocytic main processes.

Disclosures: C. Chien: None. **Y. Cheng:** None. **C. Huang:** None. **Y. Lee:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

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Program #/Poster #: 601.09

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01 Grant 0758390-06A1

Title: Optimizing ventral midbrain dopaminergic neuron differentiation through selection of Engrailed-1 expressing neuronal clusters

Authors: *A. GRAY, J. CAI, L. IACOVITTI;
Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA

Abstract: Abstract: Cell replacement therapy is a promising treatment option for Parkinson's disease due in part to the specific degeneration of ventral midbrain dopaminergic (vmDA) neurons in the substantia nigra. Stem cell therapy seeks to replace these neurons through differentiation and transplantation of induced pluripotent stem cells (iPSCs). One of the obstacles to this treatment option is the off-target differentiation of transplanted iPSCs that leads to graft

heterogeneity. Previous studies have attempted to avoid this issue by selecting neural progenitors in vitro that express markers important to vmDA lineage such as Lmx1a, Foxa2, and Otx2. However, while these markers are necessary, they are not sufficient to accurately and uniquely specify vmDA differentiation. Engrailed1 (En1) has been shown to be a reliable marker, in conjunction with Lmx1a, Foxa2, and Otx2, to successfully predict maturation of progenitors into vmDA neurons in graft. This study sought to optimize En1 expression of in vitro iPSC cultures to improve the vmDA neuron homogeneity in graft outcomes. In order to reliably determine the expression of En1 in differentiating iPSCs, an En1-tdTomato iPSC reporter line was created using Crispr-Cas9 and verified with En1/RFP co-labeling after induction of vmDA differentiation. A protocol utilizing dual SMAD inhibition, and canonical Wnt and Sonic hedgehog signaling was used to induce vmDA differentiation (similar to Gantner et al., 2020) and, after optimization, peak En1 expression was achieved at 3.5μM CHIR to adopt a midbrain fate. Differentiating our En1-Td iPSC reporter line with this protocol, we were able to see clusters of neuronal progenitors at day 20 of differentiation enriched with En1 compared to the rest of the cell culture. These En1 enriched clusters were separated from non-clustered cells using cell strainers, further cultured, and then characterized by immunostaining, PCR analysis, and single cell RNA sequencing. This data confirms that the clusters of neuronal progenitors express elevated levels of En1, along with elevated levels of Lmx1a, Foxa2, and Otx2. Selecting for these clusters in vivo could provide a simple way to filter for off target differentiation and enhance vmDA neuron homogeneity in grafts and is currently being explored in our lab.

Disclosures: **A. Gray:** None. **J. Cai:** None. **L. Iacovitti:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.10

Topic: A.03. Stem Cells and Reprogramming

Title: A scalable platform of differentiating oligodendrocyte progenitor cells in 3D suspension culture

Authors: *T. CUTIA, L. STARIKOV, A. LEPACK, A. HOVHANNISYAN, M. EBEL, N. STITT, L. SIMPSON, N. ADAMS, K. MICHEAL, D. LOSCHIAVO, E. PEREIRA, M. SRINIVAS, D. WILKINSON, J. WANG, C. PALADINI, C. PATSCH, S. IRION, J. HSU; BlueRock Therapeut., New York, NY

Abstract: Oligodendrocyte progenitor cells (OPCs) are resident glial cells in the CNS that can migrate and readily differentiate into axon-wrapping mature oligodendrocytes throughout lifetime. Emerging cell therapies leverage OPCs in treating demyelinating conditions. Derivation of OPCs from induced pluripotent stem cells (iPSCs) provides a promising platform with allogenic capability. However, major obstacles remain in transforming the production of oligodendrocyte lineage cells from petri-dish to the clinical scale (estimated $>10^8$ cells per

demyelinated lesion), complicated by a prolonged timeframe compared to most neuronal differentiations. Here we describe a comparison of two platforms that enables the production of functional OPCs in 60 days *in vitro*. First phase of dual-SMAD inhibition coupled with activation of sonic hedgehog signaling induced robust co-expression of OLIG2 and NKX2.2 in over 70% of neural progenitor cells (NPCs). These NPCs were then moved to suspension culture to form oligospheres in either stationary culture or impeller-driven mini-bioreactors for the remainder of the differentiation. We showed that oligospheres generated in bioreactors allowed for ~10-fold expansion in cell yield and improved physical uniformity of spheres. By Day 60, OPCs expressed lineage markers CD9, O4, SOX10, OLIG2, and NKX2.2. Cells generated by stationary culture and bioreactors shared remarkable similarities in protein and gene expression. A workflow was established to enzymatically dissociate oligospheres into single cells for cryopreservation. We found measurement of lactate dehydrogenase release serves a useful cell health indicator during sphere dissociation. As a result, the post-thaw viability was consistently over 70%. Lastly, we showed that the PSC-derived OPCs successfully engrafted and matured into myelin basic protein (MBP) -expressing oligodendrocytes in the hypomyelination mouse brain. Our data support the scalability of a closed system that holds great potential to meet the dose requirement for clinical applications.

Disclosures: **T. Cutia:** None. **L. Starikov:** None. **A. Lepack:** None. **A. Hovhannisyan:** None. **M. Ebel:** None. **N. Stitt:** None. **L. Simpson:** None. **N. Adams:** None. **K. Micheal:** None. **D. LoSchiavo:** None. **E. Pereira:** None. **M. Srinivas:** None. **D. Wilkinson:** None. **J. Wang:** None. **C. Paladini:** None. **C. Patsch:** None. **S. Irion:** None. **J. Hsu:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.11

Title: WITHDRAWN

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.12

Topic: A.03. Stem Cells and Reprogramming

Support: Schmidt Family Foundation

Title: Development of the Avian Telencephalic Organoid

Authors: *H. E. SCHWEIGER[1], M. A. MOSTAJO RADJI[2], M. TEODORESCU[1], M. ROLANDI[1];
[1]UC Santa Cruz, Santa Cruz, CA; [2]Genomics Inst., Univ. of California Santa Cruz, Santa Cruz, CA

Abstract: Development of avian telencephalic organoids Hunter E. Schweiger[1,2], Marco Rolandi[3], Mircea Teodorescu[1,3], Mohammed A. Mostajo-Radji[11]UCSC Genomics Institute, University of California Santa Cruz, Santa Cruz, CA, 95060[2]Department of Molecular, Cellular and Developmental Biology, University of California SantaCruz, Santa Cruz, CA, 95060[3]Department of Electrical and Computer Engineering, University of California Santa Cruz, SantaCruz, CA, 95060Brain organoids have been derived from a variety of mammalian models, including mice, human,and non human primates. Yet, the establishment of brain organoid models from non mammalianspecies has not been reported. Here we demonstrate the generation of avian telencephalicorganoids from a novel embryonic cell line, HE2M38. We derived this feeder-free cell line froma stage X chicken embryo in StemFlex medium supplemented with the WNT pathway activatorCHIR99021, the ERK pathway inhibitor PD0325901, the TGF-β pathway inhibitor A83-01, andLeukemia Inhibitory Factor (LIF).We confirmed its pluripotency over several passages byalkaline phosphatase staining and immunohistochemistry. Furthermore, we generated 3dimensional organoids and characterized the expression of canonical markers of neuronalprogenitors and neuronal subtypes in the telencephalon. Altogether, we provide a new model forlongitudinal tracking of avian telencephalic development, and a tool for comparativeneurobiology studies.

Disclosures: **H.E. Schweiger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Provisional Patent Holder. **M.A. Mostajo Radji:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Provisional Patent Holder. **M. Teodorescu:** None. **M. Rolandi:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.13

Topic: A.03. Stem Cells and Reprogramming

Support: NCATS Grant U54 TR001456
NIH Grant NS065701
NIH Grant U54 NS116025
NIH Grant NS109242
Emory UDALL Parkinson's Disease Research Center, NIH Grant P50NS123103

Title: Failure of purine recycling results in impaired energy state in developing iPSC-derived dopamine neurons

Authors: *F. SEIFAR[1], D. SUTCLIFFE[2], L. GRYCHOWSKI[2], R. FU[3], H. A. JINNAH[4]; [1]Neurosci. Grad. Program, Dept. of Neurol., [2]Dept. of Neurol., [3]Dept. of Pharmacol., [4]Dept. of Neurol. & Human Genet., Emory Univ., Atlanta, GA

Abstract: Purines are essential for ATP synthesis as the main source of energy in human cells. The adenylate energy charge (AEC) is an index of the energy status and cells maintain AEC at a narrow range regardless of ATP consumption. Two main sources of purines are de novo synthesis and recycling using hypoxanthine-guanine phosphoribosyl transferase (HGprt). Lack of HGprt is associated with abnormal human brain development seen in Lesch Nyhan disease (LND). HGprt-deficiency is linked to impaired dopaminergic development of midbrain neurons. However, the role of HGprt in brain development is still unknown. Therefore, we developed a human-derived induced pluripotent stem cell (iPSC) model to study dopaminergic neuronal development in cells lacking HGprt compared to control cells. We measured the expression of recycling and de novo purine enzymes at four different timepoints during neuronal development: iPSC, neural progenitor cell (NPC), early dopamine neuron and late dopamine neuron. We determined the level of AMP, ADP, and ATP to calculate AEC at each developmental stage. Our results revealed an increased expression of de novo purine enzymes at early development in the absence of recycling enzyme probably to compensate for lack of HGprt. However, this compensation was lost as neurons matured. There was an associated decline in AEC in HGprt-deficient neurons. Our results suggest an important role of purine recycling to maintain energy during maturation of neurons. Cells with HGprt deficiency could maintain energy status at early development by overexpression of de novo pathway. While at neuronal stage, loss of this compensation led to a decline in AEC.

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Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.14

Topic: A.03. Stem Cells and Reprogramming

Support: INPer 212250-3230-21214-03-16

Title: Effect of prolactin on differentiation of mouse embryonic stem cells to cortical neurons

Authors: *O. MARTÍNEZ-ALARCON[1], K. CARO-RODRIGUEZ[1], B. VAZQUES-MARTINEZ[1], D. COLIN-LAGOS[1], X. RAMIREZ-MEZA[1], G. CASTILLO-VILLALON[1], D. AVILA-GONZALEZ[1], G. GARCIA-LOPEZ[1], A. MOLINA-HERNANDEZ[1], W. PORTILLO[2], N. DIAZ[1];
[1]Inst. Nacional de Perinatologia, Mexico, Mexico; [2]INB, UNAM, Queretaro, Mexico

Abstract: The cerebral cortex is a central nervous system (CNS) structure generated by a constellation of interconnected neurons and glial cells to control and develop complex functions like language and memory. However, mammal development has remained uncertain due to the lack of experimental models. On the other hand, embryonic stem cells (ESC) are characterized by self-renewal and the capacity to differentiate into derivatives of the three embryonic layers. These properties offer an alternative to establishing new models in developmental biology, thus allowing the evaluation of molecules related to CNS development, such as neurotransmitters, cytokines and hormones. Interestingly, prolactin (PRL) is a hormone related to over 300 physiological functions in vertebrates, i.e. maternal behavior and adult neurogenesis. Nonetheless, its possible role in cerebral cortex embryonic development is unknown. Here, we determined the effect of PRL on the differentiation of mouse ESC to cortical neurons. To this end, we probed several hormone concentrations during the proliferation or differentiation steps of the protocol. The highest PRL concentration during proliferation generated an increasing trend of Sox2+ and Nestin+ cells, but no statistical differences were found, whereas all hormone concentrations induce an increasing trend in β-tubulin-III+ cells without reaching the statistics difference. In contrast, we found a decrease in Map2+ and NeuN+ cells with the highest and lowest concentrations, respectively. During the differentiation stage of the protocol, the treatments have no changes in any group compared to the control. These data suggest a possible PRL role during corticogenesis from mouse ESC.

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Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.15

Topic: A.03. Stem Cells and Reprogramming

Support: the Hungarian Scientific Research Foundation Grant ANN-135291

Title: Activity-dependent differentiation of human induced neurons

Authors: *A. SZÜCS[1], J. LAGERWALL[2], A. RÁTKAI[1], K. BAUER[1], K. TÁRNOK[1], K. SCHLETT[1], J. MERTENS[3,2];

[1]Dept. of Physiol. and Neurobio., Eötvös Loránd Univ., Budapest, Hungary; [2]Inst. of Mol. Biol., Univ. of Innsbruck, Innsbruck, Austria; [3]LOG-G, The Salk Inst., La Jolla, CA

Abstract: One of the intriguing questions of developmental neuroscience is whether the differentiation of neurons into distinct physiological phenotypes is primarily governed by their inherent genetic program, or the history of their electrical activity is also influential. This notion is motivated by observations demonstrating the variety of sophisticated activity-dependent mechanisms that regulate intrinsic physiological properties of neurons in the nervous system. This problem is especially intriguing in case of human neurons obtained via genetic reprogramming techniques. Conventionally, homeostatic changes in developing neurons are induced by pharmacological manipulation of their firing intensity. In the present study we used a different approach, namely, we aimed to regulate the maturation and differentiation of human induced neurons using optogenetics. First, we performed a detailed, comparative electrophysiological and immunocytochemical analysis of isogenic human neurons derived either from induced pluripotent stem cells or via direct reprogramming. Next, we elicited firing of ChR2-transduced neurons using optical stimulation of various frequency and intensity for durations of up to 48 hours. We found that such trained neurons exhibited more mature physiological properties, higher degree of intrinsic excitability and lower membrane time constant than their dark-kept controls. Hence, the stimulation was effective in speeding up the physiological maturation of the cells. Additionally, formation of synaptic connections and development of coherent network activity was facilitated by the training protocol. Our findings show that activity- and pattern-dependent regulation of the physiological properties of neurons can play a significant role in shaping their mature integrative properties.

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Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.16

Topic: A.03. Stem Cells and Reprogramming

Support: CONACYT scholarship
FODECIJAL 2021 PROYECTO 9790-2021

Title: Generation of human induced pluripotent stem cell from Mexican Alzheimer disease patient carrying the A431E mutation in the PSEN1 gen

Authors: *M. A. HERNANDEZ-SAPIENS[1], E. E. REZA-ZALDIVAR[1], A. L. MÁRQUEZ-AGUIRRE[1], J. C. MATEOS-DIAZ[2], V. SANCHEZ[3], R. CEVALLOS[4], U. GÓMEZ-PINEDO[5], J. MATIAS-GUIU[5], A. A. CANALES-AGUIRRE[1];
[1]Unidad de Evaluación Preclínica, [2]Unidad de Biotecnología Industrial, CIATEJ, Guadalajara,

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Abstract: Alzheimer's disease (AD) is a chronic brain disorder characterized by progressive cognitive decline, accompanied by memory loss and neuronal death, mainly caused by the extracellular deposition of amyloid-beta protein, and intracellular accumulation of neurofibrillary tangles (NFTs) consisting of hyper-phosphorylated tau protein mainly in brain areas, such as the cortex and hippocampus. There are two variants of AD; late-onset which affects people over 65 years old; and early-onset, which is hereditary and affects people at ~45 years old. To date, there is no cure for AD; consequently, it is essential to develop new tools to study the processes involved in the disease. Currently, the use of induced pluripotent stem cell (iPSC)-derived neurons has broadened the prospects for *in vitro* disease modeling which can be used for mechanistic studies and evaluation of therapeutic candidates in a personalized way. Therefore, this study aimed to generate iPSCs carrying the Jalisco A431E causal mutation in PSEN1. Here were generated human iPSCs from skin fibroblasts obtained from Mexican patients carrying the Jalisco A431E mutation in the PSEN1 gene, and fibroblasts without mutation. iPSCs were obtained following reprogramming using the integration-free Sendai Virus system which allows expression of the Yamanaka factors. The resulting iPSCs were expanded for ten passages to assess the stability of their self-renewal ability. iPSCs expressed pluripotency genes (OCT4, NANOG, KLF4, and SOX2) and pluripotency markers (OCT4, SOX2, and Tra-1-60). iPSCs showed the ability to differentiate into neuronal lineage as confirmed by the expression of neuronal markers such as Nestin, Sox2, and MAP2. The reported PSEN1-A431E iPSC may be used to model and study human AD pathology *in vitro* as well as the screening of compounds with therapeutic potential.

Disclosures: M.A. Hernandez-Sapiens: None. **E.E. Reza-Zaldivar:** None. **A.L. Márquez-Aguirre:** None. **J.C. Mateos-Diaz:** None. **V. Sanchez:** None. **R. Cevallos:** None. **U. Gómez-Pinedo:** None. **J. Matias-Guiu:** None. **A.A. Canales-Aguirre:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.17

Topic: A.03. Stem Cells and Reprogramming

Support: NIGMS 1P20GM134974-01A1

Title: Development of Cerebral Organoids Derived from Human Placenta Stem Cells

Authors: *C. HAACKER[1], X. TIAN[2], K. KEYS[3], J. S. ALEXANDER[4], Y. WANG[5], X. LU[6]; [1]Pharmacology, Toxicology, & Neurosci., LSU Hlth. Sci. Ctr., shreveport, LA; [2]Dept. of Pharmacology, Toxicology & Neurosci., Hlth. Sci. Ctr., Shreveport, LA; [3]Dept. of

Pharmacology, Toxicology, And Neurosci., [4]Dept. of Mol. and Cell. Physiol., [5]Dept. of Obstetrics and Gynecology and Med., Louisiana State Univ. Hlth. Shreveport, Shreveport, LA; [6]Dept. of Pharmacology, Toxicology, And Neurosci., LSU Hlth. Sci. Ctr. Cell Biol. & Anat., Shreveport, LA

Abstract: Aging-associated cognitive decline and neurodegenerative disorders are debilitating and lack disease-modifying therapy. In the last decade, stem cell therapies from autologous tissues to generate brain cells, with no ethical considerations, have attracted much scientific interest. Four characteristics make human Placental derived Stem Cells (hPSCs) “New Hope” for the clinical application of stem cells: the lack of ethical considerations, pluripotency, availability in nearly unlimited supply, and low immunogenicity. However, the significant obstacles to clinic applications of stem cell therapy include the limited capability to differentiate stem cells into neurons, poor tracking/ control over transplant cell differentiation and survival in vivo, and poor evaluation of preclinical efficacy in animal models. In order to overcome these obstacles, we have tested different serotypes and transgene promoters that could achieve more than 80% transduction efficiency with long-term stable transgene expression. We have performed transplantation of hPSCs into the striatum and substantia nigra of mice. To our surprise, we identified engrafted hPSCs with fully differentiated dendrites, axons, and dendritic spines in multiple brain regions, suggesting functional connections. Furthermore, in order to achieve a higher cell survival rate, multilineage neurodifferentiation, and robust vascularization, we established cerebral organoids from the hPSCs using a modified protocol, which yields organoids with validated morphology and cell-type composition, including neural tracer, SOX2+, and TUJ1+ cells. hPSCs-derived brain organoids were transplanted into the mouse striatum with no robust sign of astrogliosis (GFAP) or microgliosis (Iba1) around the site of implant, signifying a normal inflammatory response. The implanted cells also showed co-localization with endogenous post-synaptic cell markers (PSD95), owning to the idea that there is functional connectivity occurring between the organoid and the surrounding brain. Specifically, it was shown that there was an expression of Dopamine D_2 receptors that co-localized with organoid markers, potentially identifying those implanted organoids that can differentiate in reaction to the engrafted brain region. Together, these findings give promise for organoids as a new therapy to alleviate neurodegenerative disorders.

Disclosures: C. Haacker: None. **X. Tian:** None. **K. Keys:** None. **J.S. Alexander:** None. **Y. Wang:** None. **X. Lu:** None.

Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.18

Topic: A.03. Stem Cells and Reprogramming

Support: NARSAD 29650

Title: A pooled viral transcription factor library approach to dissect neuronal diversity

Authors: *P. MAZZARA, H. LILIOM, T. BUCKLEY, A. YONEY, K. BALDWIN; Columbia Presbyterian Med. Ctr., New York, NY

Abstract: Investigating human neuronal diversity is one of the challenges of today's neuroscience. Many neurologic diseases are linked to widely expressed genes that have effects only on rare and specific neuronal subtypes. Due to the post-mitotic nature of neurons, disease etiology and treatments have historically been explored using animal models that can poorly replicate human symptoms and progression. Using reprogramming and pluripotent stem cells it is possible to produce many human subtypes of interest for disease, such as peripheral sensory neurons, motor neurons and dopaminergic neurons. Yet, for the majority of cell types in the brain, we lack methods to identify or produce them in vitro. To overcome this problem, we have established a somatic cell reprogramming strategy to generate the whole plethora of neuronal subtypes by screening a pooled lentiviral library of transcription factors (TFs) expressed in neural lineages and involved in neuronal specification and maturation. Using a new genome edited induced pluripotent stem cell (iPSC) SNAP25-P2A-tdTomato reporter cell line, single tdTomato positive induced neuronal cells have been isolated. These induced neurons show strong morphological diversity (unipolar, bipolar and multipolar neuronal morphology) among them. Moreover, our preliminary analysis, performed using our previous published mouse neuronal TF library, show strong TFs diversity between cells and expand the list of TFs able to generate iNs in vitro. Linking the TF input to the transcriptional output signatures will expand the reprogramming toolbox and may also shed light on mechanisms underlying the generation of neuronal diversity in humans.

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Poster

601. Pluripotent Stem Cells: Differentiation and Reprogramming

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 601.19

Topic: A.03. Stem Cells and Reprogramming

Title: Transcription Factor-Based Rapid Differentiation of Human iPSCs into Inhibitory, Excitatory and Sensory Neurons

Authors: *Y. CHEHREGHANIANZABI, M. KILANDER, T. TANAKA, M. KO; Elixirgen Scientific, Elixirgen Scientific Inc., Baltimore, MD

Abstract: Ideally neuronal diseases should be studiedusing cells and tissues obtained from patients. However, there are barriers inobtaining these materials, due to their limited availability for research, andethical reasons. Additionally, the human brain structure is quite distinct fromthose of experimental animals, so animal cell-based models are not necessarilysuited for

recapitulating human physiology. Thus, there is a critical need inestablishing an accessible human cell-based disease model. To solve these problems,human induced pluripotent stem cells (hiPSCs) have been used extensively as anunlimited source of neurons of human origin. This is because when appropriatecues are provided at the right time, hiPSCs can differentiate into any type ofneurons in both the central and the peripheral nervous systems, whichintroduces hiPSC-derived neurons as an attractive resource. However, theproblem of the stepwise differentiation methods is the long duration of culturethat typically takes several weeks. To address this issue, we have developed amethod that allows us to efficiently differentiate human iPS or embryonic stem(ES) cells into a neuron subtype, such as GABAergic, excitatory, and sensoryneurons, within 10 days. This approach utilizes four serial deliveries of acocktail of synthetic messenger RNAs encoding transcription factors into hPSCs.In this manner, their expression levels can be sustained high enough to rapidlyreprogram the epigenetic marks associated with pluripotency without leavingtheir genetic footprints. In the case of GABAergic neurons, the expression ofmarker proteins such as PVALB and GAD1/GAD2 was confirmed in 74% and 65% ofTUBB3-positive cells, respectively, in the culture 10 days afterdifferentiation induction by immunofluorescence microscopy. Furthermore, GABAsecretion was detected in the culture of GABAergic neurons 17 days (282±27.0 ng/ml) and 24 days (791±75.5 ng/ml) after the initiation of differentiationby ELISA. Likewise, 57% of TUBB3 positive cells exhibited VGLUT2 expression inthe day 10 culture for excitatory neuron differentiation. With respect tosensory neuron differentiation culture on day 10, 89% of PRPH-positive neuronswere SCN9A positive. This approach has been successfully applied to severaldifferent hiPSC lines derived from healthy donors as well as from patients.Collectively, this technique is amenable to accelerate our understanding in thehuman brain development and neurological disorders. This study was conducted byour internal fund and the authors declare no financial conflict of interest.

Disclosures: **Y. Chehreghanianzabi:** A. Employment/Salary (full or part-time):; Elixirgen Scientific Inc. **M. Kilander:** A. Employment/Salary (full or part-time):; Elixirgen Scientific Inc. **T. Tanaka:** A. Employment/Salary (full or part-time):; Elixirgen Scientific Inc. **M. Ko:** A. Employment/Salary (full or part-time):; Elixirgen Scientific Inc..

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.01

Topic: A.05. Axon and Dendrite Development

Support: R15 from NICHD

Title: The role of microtubule-associated protein tau in netrin-1 attractive signaling

Authors: *T. MAJUMDER[1], B. KHOT[2], H. HUANG[3], G. LIU[1];
[2]Biol. Sci., [1]Univ. of Toledo, Toledo, OH; [3]The Univ. of Toledo, Toledo, OH

Abstract: The Role of Microtubule-Associated Protein Tau in Netrin-1 AttractiveSignalingTanushree Majumder, Bhakti Khot, Huai Huang, Guofa Liu Modulation of actin filament and microtubule (MT) dynamics in the growth cone facilitates axonoutgrowth and pathfinding in the developing nervous system. Netrin-1, a canonical guidance cue,directly regulates MT dynamics to steer growth cone navigation and promote axon outgrowth,branching, and pathfinding during development. However, how netrin-1 regulates MT dynamicsin axon turning remains a major unanswered question. The MT-associated protein (MAP) tauregulates MT stability and dynamics in neurons via regulating assembly, dynamic behavior, andthe spatial organization of MTs. Our study shows that tau directly interacts with the netrin-1attractive receptor Deleted in Colorectal Cancer (DCC), and netrin-1 induces this interaction inprimary neurons. Tau colocalizes with DCC in the growth cone of primary neurons and netrin-1induces this colocalization. Knockdown of tau not only reduces the netrin-1-induced interactionof DCC with polymerized MTs, but also inhibits netrin-1-induced axon outgrowth, branching, andcommissural axon attraction *in vitro* and leads to defects in commissural axon projection in thechick spinal cord *in vivo*. These findings suggest that the tau-dependent modulation of MTs isinvolved in netrin-1 signaling and essential for netrin-1-promoted neuronal development.This research was funded by:R15 from NICHD

Disclosures: T. Majumder: None. **B. Khot:** None. **H. Huang:** None. **G. Liu:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.02

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS062047
NIH Grant NS112504

Title: Multiple Mechanisms Guide Bifurcating Sensory Axons into the Dorsal Funiculus during Spinal Cord Development

Authors: *B. M. CURRAN, L. MA;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: The dorsal funiculus in the spinal cord is an important fiber track that relays somatosensory information to the brain. Development of this stereotypic structure involves bifurcation or generation of T-shaped central projections of dorsal root ganglion (DRG) sensory neurons at the dorsal root entry zone (DREZ), an interface between the peripheral and central nervous system. Formation of this stereotypic structure is known to be regulated by two pathways: CNP/Npr2/cGMP/PrkG1, required for forming the second branch, and Slit/Robo, required for forming the proper T-structure. However, loss of Slit or Robo only affects guidance of portion of the DRG axons at the DREZ. Here, we show the presence of additional mechanisms

for properly guiding the bifurcating axons. Wholemount characterization using neuronal and genetic markers in mice lacking the extracellular guidance molecule Netrin-1 (Ntn1) (n=5) reveals that some sensory axons escape from the dorsal funiculus during the time of bifurcation (embryonic day (E) 10.5). The misprojection defect, including fibers splaying out in random directions and defasciculation of the tight bundle, is different in their trajectories from that found previously in mice lacking Slit or Robo (Ma and Tessier-Lavigne, 2007). Furthermore, triple genetic deletion of Ntn1, Slit1, and Slit2, (n=3) leads to a completely disorganized dorsal funiculus with exaggerated misprojecting fibers in the spinal cord, demonstrating an additive effect. Single neuron analysis by lipophilic dye labeling at E12.5, revealed that loss of Ntn1 does not affect the formation of the second branch, but rather alters guidance of bifurcating axons. Individual axons (n=32) of Ntn1 mutants (n=5) showed deviation of turning angles at the branch junction and of straightness of branch trajectories. Axons (n=21) in mice lacking Ntn1, Slit1, and Slit2 (n=3) show more deviation than the single mutants alone. To determine the precise timing when Ntn1 plays its role in guidance of DRGS, we used sparse DRG specific Cre-reporter labeling of single axons at E10.5 (n=20) and found that this misguidance occurs at the time of initial bifurcation. With loss of Ntn1 during bifurcation, local turning angles were already altered in both bifurcated and pre-bifurcated axons. Based on these *in vivo* studies, we propose a complex, multi-step guidance mechanism that ensures proper formation of a seemingly simple bifurcated axonal structure during spinal cord development.

Disclosures: B.M. Curran: None. **L. Ma:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.03

Topic: A.05. Axon and Dendrite Development

Support: NINDS R37 047484
NIH

Title: The fragile x syndrome protein fmrp is required for axon guidance mediated by the wnt/planal cell polarity pathway

Authors: *P. MARFULL OROMI, K. ONISHI, Y. ZOU;
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Abstract: Planar cell polarity (PCP) pathway is known to mediate the function of the Wnt extracellular cues in growth cone guidance. Here, we show that the PCP pathway may directly influence local protein synthesis within the growth cones. Looking for Fzd3 binding partners, we found by Mass Spectrometry that Fragile-X Messenger RibonucleoProtein (FMRP) interacts with Fzd3, which was confirmed by co-immunoprecipitation. This interaction was negatively regulated by Wnt5a, which in turn induced FMRP phosphorylation and hence its activation.

FMRP is a mRNA binding protein that regulates translation and its mutation in human leads to Fragile-X syndrome and other developmental delays and cognitive deficits. Similar to some PCP components, knocking down FMRP by electroporating shRNAs into the dorsal spinal cord led to a randomization of anterior-posterior turning of commissural axons, which could be rescued by a FMRP rescue construct. Finally, we examined the presence of several FMRP target mRNAs in commissural neuron growth cones using RNAscope. We found two PCP components (PRICKLE2 and Celsr2) and other molecules regulating cytoskeletal dynamics or components of cytoskeleton (APC, Cfl1, Map1b, Tubb3 and Actb) were found in the commissural neuron growth cones using RNAscope. Our results suggest that PCP signaling may control axon guidance by regulating local protein synthesis in the growth cones through a functional interaction between Wnt/Frizzled3 and FMRP.

Disclosures: **P. Marfull Oromi:** None. **K. Onishi:** None. **Y. Zou:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.04

Topic: A.05. Axon and Dendrite Development

Support: Australian Postgraduate Awards (APA)

Title: Ten-m3 and EphA7 both play different roles in regulating the formation of topographic maps in the ipsilateral and contralateral visual circuits

Authors: *S. C.-H. LIU, A. SAWATARI, C. LEAMEY;
Fac. of Med. and Hlth., Sch. of Med. Sci., Sydney, Australia

Abstract: Because the precise alignment between contralateral and ipsilateral visual circuits is important for the development of visual system, both circuits need to be precisely regulated concurrently. Eph-ephrin and Teneurin are two important membrane protein families that are involved in regulating the development of the visual pathway (Kania & Klein, 2016; Leamey & Sawatari, 2014). Glendining et al (2017) recently showed that there is a potential interaction between Teneurin transmembrane protein 3 (Ten-m3) and Ephrin receptor A7 (EphA7) as the expression of EphA7 was reduced in Ten-m3 KO.
In order to investigate the roles of EphA7 and Ten-m3 on regulating the formation of ipsilateral and contralateral patterns of retinal ganglion cell (RGC) innervations (topographic maps) in the visual system, we injected various tracer dyes, e.g. DiI, CTb and WGA-HRP, into the retina, eyeball and dorsal lateral geniculate nucleus (dLGN) of the postnatal day 13 EphA7 KO and Ten-m3 KO mice respectively.
Our results showed that EphA7 KO had mild effects on the development of the ipsilateral RGCs (ipsilateral projections) from ventral retina, while Ten-m3 KO had greater effects on the ipsilateral RGCs from temporal retina. The results pointed to the complementary roles for Ten-

m3 and EphA7 in regulating the development of ipsilateral projections in the visual system. In terms of patterning of contralateral projections in the visual system, similar topographic errors were found from different subsets of the contralateral RGCs in Ten-m3 KO and EphA7 KO mice, suggesting that they might have similar functional roles in regulating the development of specific contralateral RGCs. Our finding demonstrated that Ten-m3 and EphA7 both may play different roles in regulating the formation of the ipsilateral and contralateral topographic maps in the visual system. Behavioural studies revealed a potential deficit in the ability of EphA7 KO mice to discriminate between visual stimuli located in their dorsal visual field, suggesting the functional importance of EphA7 in visual behaviors.

Disclosures: S.C. Liu: None. **A. Sawatari:** None. **C. Leamey:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.05

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant GM083889
NIH Grant MH120414
NIH Grant 1T32GM135060-01

Title: Fascin1-actin regulation of axonal development and brain wiring

Authors: *K. HARDIN[1], C. YE[1], K. H. MOBERG[1], K. R. MYERS[1], J. Q. ZHENG[2];
[1]Cell Biol., [2]Cell Biology, Ctr. for Neurodegenerative Dis., Emory Univ., Atlanta, GA

Abstract: Axon guidance is a critical developmental process in which axonal projections are guided by the tips of axons, called growth cones, to their specific targets for the precise wiring of the central nervous system. Errors in axon guidance can result in wiring defects that are associated with a wide range of brain disorders including autism and epilepsy. Axonal growth cones are powered by two distinct actin-rich membrane protrusions, lamellipodia and filopodia. Filopodia are finger-like protrusions supported by bundled actin filaments that sense a wide range of extracellular guidance cues. The pharmacological elimination of growth cone filopodia results in axon guidance defects without impeding axon elongation. However, the molecular and cellular mechanisms that regulate filopodia dynamics and their responses to guidance cues remain unclear.
Fascin1 is a ~55 kDa actin bundling protein that crosslinks actin filaments to form tight F-actin bundles in filopodia and is a known regulator of cell migration. Fascin1 is highly expressed in developing neurons and enriched in growth cone filopodia, but its role in growth cone motility and guidance has not been investigated. Here, we use a combination of cell culture and *in vivo* approaches to demonstrate the role of Fascin1 in growth cone filopodia dynamics, motility, and axon guidance. Using a novel CRISPR-Cas9-mediated knockout approach in cultured primary

cortical neurons, we show that Fascin1 knockout results in a marked reduction in axonal elongation and branching. In addition, we demonstrate how the loss of Singed, the *Drosophila melanogaster* ortholog of Fascin1, affects *in vivo* brain wiring and function in fruit flies. Specifically, we found that *singed* null flies exhibit marked axonal defects in the mushroom body, a brain structure that is analogous to the mammalian hippocampus. Together, our work highlights the important role of Fascin1 in actin-based axon development and brain wiring. Additionally, the use of both a cultured cell system and an *in vivo* model allows us to examine the neurodevelopmental function of Fascin1 at both the single cell and organismal levels.

Disclosures: K. Hardin: None. **C. Ye:** None. **K.H. Moberg:** None. **K.R. Myers:** None. **J.Q. Zheng:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.06

Topic: A.05. Axon and Dendrite Development

Support: NINDS Intramural Research Program 1ZIANS003140-08

Title: The Neuronal Rho-GEF Trio mediates BDNF-TrkB stimulated neurite outgrowth & complexity

Authors: A. LOMBARDO[1], E. FINGLETON[1], Y. LI[2], *K. ROCHE[1];
[2]Proteomics Core Facility, [1]NINDS, NIH, Bethesda, MD

Abstract: Trio is a neuronal Rho Guanine nucleotide Exchange Factor (GEF) critical for Rac1 and RhoA activation and thereby modulates actin polymerization, cytoskeleton remodeling, and neuronal development. Recent findings discovered an Autism Spectrum Disorder (ASD)-related de novo mutation hotspot within Trio's Rac1 activating domain, GEF1. Previously, we identified TrkB, the brain-derived neurotrophic factor (BDNF) receptor, as a Trio interactor via affinity purification-mass spectrometry. Activation of the BDNF-TrkB pathway is crucial for regulating neuronal morphology and is implicated in various neurodevelopmental disorders, including ASD and Intellectual Disability. Given that Trio is essential for Rac1 activation and interacts with TrkB, Trio may be critical to synaptic plasticity mediated by the BDNF-TrkB pathway. While BDNF has been shown to act through other Rho-GEFs, it is unknown whether a BDNF-TrkB-Trio pathway exists. Our preliminary data and the functional overlap between Trio and BDNF-TrkB signaling led us to hypothesize that Trio promotes Rac1 activation and cytoskeletal remodeling downstream of TrkB activation during neurite outgrowth. Using short hairpin RNA, we knocked-down Trio in primary hippocampal neurons and bath applied BDNF. We observe that BDNF treatment induces an increase in neurite outgrowth and complexity, as previously described, but not in Trio knock-down neurons, suggesting Trio is necessary for BDNF-TrkB stimulated neurite outgrowth. Future studies will address the downstream effectors of Trio, such

as Rac1, and whether this pathway is invoked in contexts outside of dendritic patterning. A concrete understanding of the functional importance of Trio will provide insight as to how Trio's disruption contributes to the pathogenesis of several neurodevelopmental disorders and may illuminate novel therapeutics for individuals possessing Trio mutations.

Disclosures: A. Lombardo: None. **E. Fingleton:** None. **Y. Li:** None. **K. Roche:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.07

Topic: A.05. Axon and Dendrite Development

Support: 1ZIANS003140-08

Title: Trio and CRMP2 interact to regulate axon morphology

Authors: *E. FINGLETON[1,3], A. LOMBARDO[1], Y. LI[2], K. W. ROCHE[1];
[1]RBS/NINDS, [2]Proteomics Core Facility, NINDS, NIH, Bethesda, MD; [3]Neurosci. Dept., Brown Univ., Providence, RI

Abstract: Trio is a neuronal Rho Guanosine nucleotide Exchange Factor (GEF) that is critical for typical neurodevelopment: mutations in Trio's GEF domains are associated with profound neurodevelopmental disease, and neuronal knock-out of TRIO in mice results in gross anatomical defects and perinatal lethality. Axon guidance is an important process during nervous system development through which various neuronal populations are guided to innervate their correct anatomical targets. Although multiple studies have identified a role for Trio in axon guidance, the mechanism regulating Trio activation in these contexts remains unclear. Through affinity purification-mass spectrometry our lab identified several Trio interactors, including Collapsin Response Mediator Protein 2 (CRMP2), which mediates various axon guidance signaling events. Through co-immunoprecipitation, we verified the CRMP2/Trio interaction in vivo. To understand the functional effect of the interaction, we used short hairpin RNA (shRNA) to knock-down Trio in primary hippocampal neurons and activated the CRMP2 growth cone collapse pathway through either Semaphorin3A (Sema3A) bath application, or overexpression of phosphomimetic CRMP2. Overall, our results indicate a critical role for Trio in mediating axon repulsion and suppression of axon branching downstream of Sema3A and CRMP2. Unexpectedly, activation of the repulsive CRMP2 pathway promotes axon branching in Trio knock-down neurons. Future studies on the downstream effectors of this pathway will both grant insight into the CRMP2/Trio interaction and enhance our understanding of the mechanisms of repulsive axon guidance. Additionally, elucidating Trio's role in axon guidance will advance our understanding of neurodevelopment and may yield clinical interventions for patients with mutations affecting Trio. This work is funded by the NIH Intramural Research Program under grant 1ZIANS003140-08

Disclosures: **E. Fingleton:** None. **A. Lombardo:** None. **Y. Li:** None. **K.W. Roche:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.08

Topic: A.05. Axon and Dendrite Development

Support: NIH R01 NS Grant
SFARI Pilot Award

Title: Loss of ASD gene Bcl11a causes aberrant callosal projection neuron projections into the basolateral amygdala and leads to abnormal behaviors

Authors: *J.-Y. KIM[1], O. DURAK[1], M. WETTSTEIN[3], J. D. MACKLIS[2];
[1]Harvard, [2]Harvard Univ., Harvard Univ., Cambridge, MA; [3]Cornell Univeresity, Ithaca, NY

Abstract: Neocortex and its diversity of neuronal subtypes processes sensorimotor and associative integration, with higher-order cognition via precise subtype-specific connections. Central in associative integration are interhemispheric callosal projection neurons (CPN) that connect the cerebral hemispheres via the *corpus callosum* (CC). Diverse, yet precise, connectivity of areally diverse CPN is critical for sensory, associative, and behavioral functions, with CPN centrally implicated in autism spectrum disorder (ASD) and intellectual disability. CPN development is regulated by multiple TFs, among which *Bcl11a/Ctip1* regulates multiple core aspects of CPN development, including subtype specification, cortical areal organization, and precision of CPN axonal targeting (e.g. Woodworth, Greig, *Cell Rep*, 2016; Greig, Woodworth, *Neuron*, 2016). In humans, *BCL11A* is recently identified as a monogenic, causal ASD/ID gene; patients with missense mutations exhibit autistic features and intellectual disability. Here, we identify multiple remarkable circuit abnormalities relevant to ASD upon Bcl11a/Ctip1 mutation. First, forebrain-specific deletion of *Bcl11a* causes loss of precise homotopic contralateral hemisphere targeting, and dramatically increases the number of axons projecting through the anterior commissure in adult mice. Even more strikingly, *Bcl11a*-null sensorimotor CPN establish aberrant and otherwise absent projections specifically into basolateral amygdala, well-studied re: fear, anxiety, and centrally implicated in ASD. *Bcl11a cKO* mice exhibit disrupted exploratory and cognitive behaviors. Thus, *Bcl11a/Ctip1* mutation dramatically and specifically disrupts precision of axonal targeting during development, leading to remarkably aberrant circuits- in particular from sensorimotor cortex into the basolateral amygdala, promiscuous contralateral targeting, and aberrant projection through the anterior commissure- along with highly ASD-relevant abnormal behaviors. *Bcl11a* serves as an exemplar to investigate molecular controls over functional associative cortical circuitry, and the dysfunctions of subcellular molecular machinery potentially underlying development of ASD/ID.

Disclosures: **J. Kim:** None. **O. Durak:** None. **M. Wettstein:** None. **J.D. Macklis:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.09

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS116463
NIH P20GM103440
NIH P20GM103650

Title: Molecular mechanism underlying axonal localization of Dual Leucine Zipper Kinase in Drosophila

Authors: *S. KIM, M. SINGH, J. KIM;
Dept. of Biol., Univ. of Nevada, Reno, NV

Abstract: Proper protein localization is essential for many biological functions. Axonal transport is a process that enables spatial and temporal control of the protein localization in neurons. Dual Leucine Zipper Kinase (DLK) is an axonal protein and mediates multiple stress signals in axon development, axon regeneration, and neuronal cell death. However, the molecular mechanism underlying the axonal localization of DLK is not known. In this study, we found that Wallenda (Wnd), *Drosophila* ortholog of DLK, is highly enriched in the axon terminals of *Drosophila* sensory neurons and that the two regions of Wnd mediate axonal localization – a palmitoylation site at amino acid (aa) -130 and the 201-300 aa region of Wnd. Wnd exhibits a punctate pattern in the neuronal soma, which likely represents axonally targeted vesicles. Some Wnd puncta also show a partial co-localization with the somatic Golgi, which suggests that the sorting process of Wnd occurs in the somatic Golgi. Furthermore, we found that a palmitoylation-defective Wnd showed almost exclusive localization on the somatic Golgi where it extensively colocalizes with the *Drosophila* Huntingtin-interacting protein 14 (dHIP14), an ortholog of mouse HIP14 that is known to mediate DLK palmitoylation in the mouse. Interestingly, deletion of Wnd 201-300 aa completely abolishes somatic Golgi localization and redirects Wnd to the somatic plasma membrane with greatly reduced axonal localization. These suggest that the axonal anterograde transport of Wnd takes multiple steps: the initial Golgi localization of Wnd that requires Wnd 201-300 aa, dHIP14-mediated palmitoylation on Golgi, and the packaging and sorting of Wnd into axonally targeted vesicles. As a critical kinase in stress signaling, Wnd protein levels are kept low by Highwire (Hiw), an evolutionarily conserved E3 ubiquitin ligase. We found that Wnd down-regulation by Hiw occurs in the axon terminals, but not in the cell body. These results suggest that the location of palmitoylation determines DLK's axonal localization and that DLK is actively transported out of the neuronal cell body for Hiw-mediated suppression in the axon terminals. Our findings uncover novel mechanisms of Wnd transport and provide insights into how axonal transport is coupled to regulated protein turnover.

Disclosures: S. Kim: None. **M. Singh:** None. **J. Kim:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.10

Topic: A.05. Axon and Dendrite Development

Support: DP2MH122398

Title: The novel gene Ganon-1 produces a scaffolding RNA for growth signaling in developing axon projections.

Authors: *G. W. BUNCE, A. ROMANOWSKI, C. ROBERTSON, C. BRANDENBURG, R. RICHARDSON, B. ALTAS, A. POULOPOULOS;
Univ. of Maryland: Baltimore, Univ. of Maryland: Baltimore, Baltimore, MD

Abstract: The brain develops high complexity by integrating information from the environment and the entire genome, including its vast non-protein-coding regions. We identify here a novel gene product that does not encode protein and is enriched as a processed RNA in axon growth cones of cortical projection neurons in the developing mouse brain. We name this non-coding RNA (ncRNA) Ganon-1, and demonstrate that it has hybrid molecular features between mRNAs and ncRNAs. Cytosolic Ganon-1 is polyadenylated and 5' processed to reveal a canonical Terminal OligoPyrimidine (TOP) motif; the most studied mRNA translation cis-regulatory element, and a characteristic feature of axon-enriched mRNAs. Despite these features, Ganon-1 does not contain conserved open reading frames (ORFs) and is predicted to display structural intramolecular base-pairing resulting in a thermodynamically stable structure. Functional TOP motifs in mRNA transcripts bind mTOR complex 1 (mTORC1) and confer all-or-none translational dependence on mTOR signaling. We demonstrate that Ganon-1 has a bona fide TOP motif able to bind mTORC1 in early postnatal brain when mTORC1 is enriched in axon growth cones, suggesting that Ganon-1 may be a ncRNA component of putative axon mTOR outposts. We explore the expression patterns and subcellular localization of Ganon-1 and use CRISPR knockout to investigate its function in cortical axon development and in the subcellular holocomplexes of mTOR pathway proteins and RNA that comprise axon mTOR outposts. This study identifies the first non-coding RNA to bind the mTOR complex, indicating that the TOP motif is not a translation regulator, but rather an mTORC1-binding-motif of mRNAs and ncRNAs. These findings reveal an unexplored avenue in mTOR biology and axon growth with potential promise for axon regeneration.

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Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.11

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R21NS111991
2T32GM007055-44
F31NS126020
NIH Grant G20-RR31199

Title: Functional Characterization of Extracellular Vesicles in the Developing Peripheral Nervous System

Authors: *A. MASON[1], A. KEELER[2], B. WINCKLER[3], C. DEPPMANN[2];
[1]Neurosci. Grad. Program, [2]Biol., [3]Cell Biol., Univ. of Virginia, Charlottesville, VA

Abstract: Proper wiring of the peripheral nervous system relies on neurotrophic signaling via nerve growth factor (NGF). NGF secreted by target organs (i.e. eye) binds to the TrkA receptor expressed on the distal axons of postganglionic neurons. Upon binding, TrkA is internalized into a signaling endosome and retrogradely trafficked back to the soma and into the dendrites to promote cell survival and postsynaptic maturation, respectively. Postganglionic neurons that do not receive sufficient amounts of NGF will die off. Interestingly, the survival of presynaptic preganglionic neurons residing in the spinal cord is a corollary of NGF-dependent postganglionic survival. In the final circuit, preganglionic neurons are quantitatively matched to postganglionic neurons and, by extension, to the final target, even though TrkA is not expressed on preganglionic neurons. The trophic cue governing this presynaptic survival matching has yet to be identified. Here, we propose that extracellular vesicles (EVs) mediate this trophic cue between postganglionic and preganglionic neurons. Using the mouse superior cervical ganglion (SCG) as a model, we have isolated EVs and confirmed their identity, morphology, and concentration using western blot, cryoelectron microscopy and nanoparticle tracking analysis. Furthermore, we have shown that EVs secreted from SCG neurons contain retrogradely trafficked TrkA that originated in the distal axon. Lastly, using a survival assay we have shown that the addition of SCG-derived EVs to developing spinal cord cells promotes their survival and this affect is abrogated by blocking TrkA EV signaling using an anti-NGF neutralizing antibody. These data point to novel route of neurotrophic signaling through EVs. Ongoing work seeks to identify the cell types that internalize sympathetic EVs as well as the signaling pathways elicited in recipient cells.

Disclosures: **A. Mason:** None. **A. Keeler:** None. **B. Winckler:** None. **C. Deppmann:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

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Program #/Poster #: 602.12

Topic: A.05. Axon and Dendrite Development

Support: MOST 110-2628-B-A49-001
Smart Platform of Dynamic Systems Biology for Therapeutic Development

Title: The centrosomal protein Cep170 exhibits distinct non-centrosomal localization during neuronal development

Authors: *P.-T. CHEN[1,2], A. GOH[2], H.-C. HUANG[1], E. HWANG[2,3,1,4];
[1]Inst. of Mol. Med. and Bioengineering, [2]Dept. of Biol. Sci. and Technol., [3]Inst. of Bioinformatics and Systems Biol., [4]Ctr. for Intelligent Drug Systems and Smart Bio-devices (IDS2B), Natl. Yang Ming Chiao Tung Univ., Hsinchu, Taiwan

Abstract: Microtubules are essential cellular polymers in neurons and participate in essentially every step of the neuronal development. The formation and regulation of microtubules are controlled by microtubule-associated proteins (MAPs) that also play essential roles in the development of the nervous system. Using quantitative proteomics to compare MAPs from undifferentiated stem cells and stem cell-derived neurons, we found that Cep170 is more enriched on neuronal microtubules. Cep170 is a forkhead-associated (FHA) domain-containing centrosomal protein and localizes to the subdistal appendage of the centriole in mitotic cells. Its mutations have also been found to be associated with human brain abnormalities, such as microcephaly and lissencephaly. To understand the role of Cep170 in neurons, loss- and gain-of-function experiments were conducted. Overexpressing Cep170 promotes axon and dendrite elongation in both stem cell-derived neurons and primary neurons. Surprisingly, no morphological phenotypes can be observed when Cep170 is depleted. In addition, we observed that Cep170 exhibits distinct localizations in neurons: 1) at the centrosome, 2) as discrete puncta along the axon and dendrite, 3) enriched at the tip of the axon. To understand how these distinct localizations are established, different truncations of Cep170 which compromise specific localizations were overexpressed in neurons. The punctate distribution along the axon and dendrite requires both the microtubule-binding and the FHA domain, while the axon tip enrichment depends on the FHA domain. Furthermore, we discovered that microtubule stability affects the punctate distribution of Cep170 along the axon and dendrite but not the enrichment at the axon tip. Finally, we will also present data showing how Cep170 localizes to these non-centrosomal regions.

Disclosures: **P. Chen:** None. **A. Goh:** None. **H. Huang:** None. **E. Hwang:** None.

Poster

602. Axon Growth, Guidance, and Signaling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 602.13

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

Support: NIH Pioneer Award DP1 NS106665
Allen Frontiers Group
Fondation Jean-Jacques et Felicia Lopez-Loreta pour excellence académique
Travis Roy foundation
SNF postdoctoral fellowship

Title: Subcellular transcriptomes of corticospinal growth cones are distinct from transcriptomes of their somata, and locally implement development of corticospinal circuitry

Authors: *A. K. ENGMANN, P. NANDA, O. DURAK, D. NGUYEN, M. VICENT, J. D. MACKLIS;
Dept of Stem Cell and Regenerative Biology, and Ctr. for Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Corticospinal neurons (CSN) are crucial for fine motor control. Their cell bodies are located in layer V of neocortex, and during development they send their axonal projections over remarkably long distances to the brainstem and spinal cord, generating exquisitely precise, target-specific functional circuitry. Injury or degeneration of CSN circuitry causes critical loss of motor function in traumatic injury and motor neuron diseases, so understanding CSN development is likely to contribute importantly to CSN circuit regeneration and/or prevention of degeneration. Deep investigation of subtype-specific soma transcriptomes has provided substantial insight into the "molecular logic" of CSN subtype specification, development, and diversity through early-mid corticogenesis. However, knowledge of molecular mechanisms controlling CSN axon elongation, grey matter innervation, branching and collateralization, synapse formation, and ultimate circuit generation, is still quite limited. Recent work reveals that neurons (and likely other polarized cells) contain multiple distinct subcellular transcriptomes, and that local protein synthesis of axonally-enriched transcripts is required for directional responses to at least some guidance cues as well as the formation of presynaptic terminals. However, the composition of subtype-specific GC-localized molecular machinery, and the dynamic regulation of these subcellular processes, are essentially unknown. We apply a combination of subtype-specific labeling, biochemical fractionation, and subcellular fluorescence activated sorting to purify CSN-specific GCs and parent somata directly from the mouse brain. Our investigation of subcellular CSN transcriptomes identifies subsets of GC- vs. soma-localized transcripts, and identifies broad categories and specific elements of subtype-specific subcellular machinery central to generation of functional circuitry. Further, we identify that GC-localized transcriptomes are established by both RNA trafficking and transcriptional mechanisms, and that subcellular transcriptome composition is dynamically regulated across developmental stages, likely reflecting active stage-specific trafficking and local enrichment of context-specific molecular machinery to enable proper axon guidance, synaptic target selection, and circuit formation. Increasingly deep knowledge of molecular processes central to correct CSN circuit development promises to also ultimately enable understanding of mechanisms resulting in degeneration or failed regeneration following injury, and potentially lead to development of new therapeutic approaches.

Disclosures: **A.K. Engmann:** None. **P. Nanda:** None. **O. Durak:** None. **D. Nguyen:** None. **M. Vicent:** None. **J.D. Macklis:** None.

Poster

603. Synapse Maturation and Remodeling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 603.01

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

Support: 1R15 AG060461-(01)

Title: Psd95 content at young adult or juvenile hippocampal or neocortical synapses is not altered by transgenic expressing of chimeric glun2 subunits

Authors: *G. A. WILD[1], R. E. KEITH[2], J. I. ESCOBAR[2], P. P. BOUTE[1], T. C. DUMAS[1]; [1]Psychology, [2]Neurosci., George Mason Univ., Fairfax, VA

Abstract: N-methyl-D-aspartate receptors (NMDARs) are required for activity-dependent synaptic plasticity that subserves the construction of neural networks during development and the processing of information in more mature animals. The composition of NMDARs is altered in various brain structures during postnatal development in relation to alterations in learning and memory abilities. All NMDARs consist of two obligatory GluN1 subunits and two auxiliary subunits. In the mouse hippocampus, the auxiliary GluN2 subunits are primarily GluN2A and GluN2B. Most hippocampal NMDARs contain GluN2B subunits in the early postnatal period until the end of the third postnatal week, when NMDARs with GluN2A subunits supersede those with GluN2B. This GluN2 subunit switch alters ion conductance, anchoring, and direct intracellular protein signaling. Thus, to understand the importance of the GluN2 subunit switch for hippocampal development requires investigation of separate contributions of ion conductance and direct intracellular signaling. For this, we created transgenic mice expressing GluN2 chimeric subunits that express the transmembrane domains (TMDs) and N-terminus of GluN2A fused to the C-terminus of GluN2B (referred to as “ABc” mice) or vice versa, the TMDs and N-terminus of GluN2B fused to the C-terminus of GluN2A are used (“BAc” mice). Prevention of the developmental increase in the NMDAR anchoring protein, postsynaptic density protein 95 (PSD95), prevents the GluN2 subunit switch during postnatal hippocampal development. We investigated whether PSD95 expression levels at hippocampal synapses were altered in the ABc and BAc transgenic mice. We previously applied immunohistochemistry in brain sections from young adult subjects and demonstrated that the ABc mice contain increased levels of PSD95 in area CA1 and the dentate gyrus when compared to age-matched wildtype littermates or BAc mice. Using more sensitive Western Blots, we sought to confirm these differences in PSD95 levels within our chimeras in young adults and better understand the age at which the increase in PSD95 occurs. Initial data from our lab across three age groups suggest that there are no significant differences in PSD95 levels in either the adult or developmental animals in either the neocortex or hippocampus. Thus, expression of ABc or BAc subunits does not alter PSD95 expression levels, suggesting that the GluN2 subunit switch occurs in these transgenic lines. We will extend this research to native GluN2 subunits.

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Poster

603. Synapse Maturation and Remodeling

Location: SDCC Halls B-H

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Program #/Poster #: 603.02

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

Support: MRC-Sackler funded ST11869

Title: Mapping the emergence of a subcellular balance between excitatory and inhibitory synapses along dendrites

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Abstract: Understanding the spatial organisation of synapses is essential for comprehending how neurons integrate and compute information. The aim of this project was to map the distribution of excitatory and inhibitory (E/I) synapses along pyramidal cell dendrites in the hippocampus throughout development. Our goal was to uncover the logic of how E/I synapses are arranged and chart the emergence of a balance between the two. We used fibronectin intrabodies (FingRs), expressed in individual pyramidal neurons and delivered by in utero electroporation, to fluorescently label E/I postsynaptic compartments. Confocal microscopy of large, tiled image series was used to reconstruct the entire basal dendrites of pyramidal neurons in CA1 of the hippocampus at three developmental periods: P7, P14 and P21. Paired with the simultaneous labelling of E/I synapses via FingRs, this approach enabled us to map the spatial distribution of synapses across complete dendritic branches of pyramidal neurons in CA1 throughout development. We used serial block face scanning EM (SBFSEM) at these same developmental periods to assess synaptic distribution with high resolution. Confocal imaging of FingRs along basal dendrites revealed a balance between excitation and inhibition within short stretches of dendrite, down to 9µm (n=2112 excitatory and 324 inhibitory synapses across 14 dendrites from 4 cells, $p<0.05$, Spearman's rank). SBFSEM confirmed this sub-branch balance (n=889 excitatory and 75 inhibitory synapses from 8 dendrites, $p<0.05$, Spearman's rank). At P14, a period of marked synaptogenesis, the density of excitatory synapses was half of that measured at P21, although inhibitory synapses were already present at a similar density, suggesting clear differences in the timeline of synapse formation for each synapse type. Regardless of this difference, we observed a similar sub-branch balance between the excitation and inhibition already present (n=659 excitatory and 280 inhibitory synapses across 18 dendrites from 3 cells, $p<0.05$, Spearman's rank). We are currently establishing the rules of synaptic organisation at earlier timepoints within CA1 using confocal imaging of FingRs and SBFSEM. Together, our results indicate that the organisation of excitation and inhibition throughout the dendritic arbours of hippocampal pyramidal neurons is non-random and established early in development. Specifically, our results demonstrate that excitation and inhibition are proportional to one another at a sub-branch level. Basal dendrites of CA1 pyramidal neurons are known to

integrate synaptic inputs locally and our findings suggest that they are well equipped to do so in a balanced manner.

Disclosures: S. Horton: None. **G. Neves:** None. **J. Burrone:** None.

Poster

603. Synapse Maturation and Remodeling

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

Support: F32 NS106732 (NINDS, CRB)
I01BX002949 (VA, ES)
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R21 NS102948 (NINDS, IK/ES)
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P30 NS061800 (NIH, SKP)

Title: Impact of mossy cell ablation on adult hippocampal neurogenesis

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Abstract: The neurogenic niche in the subgranular layer generates new dentate granule cells (DGCs) for the hippocampal circuit even into adulthood, and circuit integration of new DGCs contributes to learning and memory. Disruption of adult-born DGC proliferation and integration occurs in various models of brain injury, coincident with hippocampal circuit changes and prominent hilar mossy cell loss. We hypothesized that activity of hilar mossy cells influences early survival and integration of adult-born DGCs, and that the loss of mossy cells following brain injury contributes to aberrant neurogenesis and integration of new neurons. To test this idea, we selectively ablated hilar mossy cells via viral-mediated (AAV5-flex-taCasp3) apoptosis in Crlr-Cre (Calcitonin receptor-like receptor) mice, which results in extensive (>70%) loss of hilar mossy cell bodies and their calretinin-positive axons in the inner molecular layer (IML) two weeks after virus injection. Microglia accumulated in the dentate gyrus at two weeks following virus injection, but neuroinflammation resolved one week later. At 3 weeks after taCasp3 virus injection, we examined the proliferation, survival, and morphologic maturation of adult-born DGCs using BrdU immunohistochemistry and retroviral injections to label adult-born DGCs. Although mossy cell loss did not impact proliferation or survival of adult-born DGCs, dendritic outgrowth was accelerated, reaching similar levels to controls at maturation. Interestingly, despite the loss of mossy cell axons in the IML, dendrites in this proximal dendritic region had an equivalent density of dendritic spines as control mice. Our preliminary data suggests that this

may reflect proximal shifting of excitatory inputs from the medial entorhinal cortex. VGlut2, which normally immunolabels the perforant path in the outer 2/3rds of the molecular layer but spares the IML, now extended to label the full width of the molecular layer in mossy cell ablated mice. Electrical stimulation of the IML also indicated the presence of functional inputs with altered E:I balance and reduced sensitivity to cannabinoid receptor agonists, consistent with structural reorganization of the molecular layer. Our results demonstrate that removal of hilar mossy cells transiently accelerates outgrowth of adult-born DGCs, and that glutamatergic inputs from the perforant path may compensate for the loss of mossy cell inputs.

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Poster

603. Synapse Maturation and Remodeling

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Program #/Poster #: 603.04

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

Support: NIH Grant R01 MH109165
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Title: Understanding Novel Functions of IL-1 and IL-1R1 Signaling in Neurodevelopment

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Abstract: The signaling system between interleukin-1 (IL-1) and its receptor interleukin-1 receptor-1 (IL-1R1) has critical roles in maintaining the homeostasis of the central nervous system (CNS), neuroinflammation, and neural circuit functions. Recent studies showed that neuronal IL-1R1 (nIL-1R1) is important for cognition and social interaction in adult mice. The role of nIL-1R1 in neurdevelopment and related cognitive and affective functions is unknown. Here, we investigated the spatiotemporal patterns of nIL-1r1 expression during neurodevelopment and examined how nIL-1R1 might modulate neurodevelopment. Using our global IL-1R1 reporter mouse line as well as lines in which we restricted the expression of IL-1R1 to specific neuronal or non-neuronal cell types, we found that nIL-1R1 expression is very dynamic during brain development and changes dramatically in various brain regions (dentate gyrus of hippocampus (DG), dorsal raphe nucleus (DRN), ventral posteromedial (VPM), and posterolateral thalamic nuclei (VPL)) between postnatal days 3-21. These changes occurred anatomically in brain regions related to neurocircuits involved in the memory, sensory, and

mood-regulation functions of the brain. In addition, we found that endothelial cells and leukocyte IL-1R1 expression modulate nIL-1R1 expression during development. Application of maternal separation as a neonatal stressor, we discovered a nIL-1R1-dependent acceleration of neuronal maturation in DG, somatosensory cortex, and frontal cortex on postnatal day 7 (P7) which was terminated by P14. These findings suggest that nIL-1R1 is tightly regulated during brain development and has a critical role in the control of neuronal maturation in early life.

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Poster

603. Synapse Maturation and Remodeling

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

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Title: Functional development of reciprocal inhibitory feedback between A17 amacrine cells and rod bipolar cells in mouse retina

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Abstract: Sensory experience during postnatal development induces architectural and functional remodeling of synaptic connections. In the rodent retina, eye opening (~postnatal day 14), drives a series of changes in circuitry development and synaptic receptor function. In the rod pathway, which mediates dim light vision, rod bipolar cell (RBC) terminals provide glutamatergic inputs to GABAergic A17 and glycinergic AII amacrine cells. While AIIs transfer signals to ON and OFF pathway, A17 amacrine cells make reciprocal inhibitory feedback synapses onto the same RBC axon terminal, modulating the time course of visual signaling *in vivo*. Although glutamate release from RBCs is known to be functional before eye opening, whether reciprocal inhibitory feedback from A17 amacrine cells is stablished prior eye opening and how sensory experience might influence its function remains unclear. Here, using whole-cell voltage clamp recordings and pharmacological approaches in acute retinal slices from young (P10-P13) and adult (P20-P40) mice of either sex, we found that the reciprocal GABAergic feedback -triggered by Ca^{2+} influx through Ca^{2+}-permeable AMPAR receptors (CP-AMPARS) in adult retina- was completely absent prior to eye opening. GABA-induced currents and glutamate-evoked inhibitory postsynaptic currents (IPSCs) suggest that GABARs at the RBC terminals are

functional prior eye opening. However, the A17 AMPAR current-voltage relation was more linear prior to eye-opening compared to adult mice, suggesting that AMPARs properties change from Ca^{2+}-impermeable AMPARs to CP-AMPARS during development. Consistently, reciprocal IPSCs were observed prior eye opening in RBCs recorded from transgenic $GluA2^{-/-}$ mice, where Ca^{2+}-influx through CP-AMPARS is boosted. Interestingly, reciprocal feedback inhibition was reduced in RBCs from adult dark-reared mice when compared to age-matched controls. Moreover, brief light-exposure (1 h; ~60 lux) following dark-rearing significantly increased the number of RBCs expressing functional reciprocal feedback compared to dark-reared mice. Altogether, our results suggests that eye opening or light experience promotes the functional establishment of reciprocal feedback at the RBC-A17 synapse, likely by changing AMPAR function in A17 amacrine cells.

Disclosures: S.F. Estay: None. **W.N. Grimes:** None. **J.S. Diamond:** None. **A.E. Chavez:** None.

Poster

603. Synapse Maturation and Remodeling

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

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Title: Characterization of synaptic pathology in $Ca_V1.4$-deficient human retinal organoids

Authors: *S. P. BHARATHAN[1,2], C. FREITAS[1], A. FERRARIO[1], K. STEPANIAN[1], G. FERNANDEZ[2], M. W. REID[1], J. G. APARICIO[1], A. NAGIEL[1,2,3];
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Abstract: *Background:* One of the earliest manifestations of blinding retinal diseases is the withdrawal of synapses at the outer plexiform layer (OPL) between photoreceptors and bipolar cells. The mechanisms underlying this synaptic miswiring are poorly understood, and it is unclear whether this remodeling is reversible upon treatment with gene therapy. Humans with congenital stationary night blindness (CSNB) due to pathogenic *CACNA1F* variants exhibit a relatively mild phenotype with evidence of primarily synaptic pathology on electroretinography

with minimal or no loss of photoreceptor cells, and hence this disease may serve as a model to dissect synaptic miswiring in other retinal diseases. The *CACNA1F* gene codes for the L-type voltage-gated calcium channel $Ca_V1.4$ required for entry of Ca^{2+} ions at photoreceptor synapses and for glutamate release onto bipolar cells in the OPL. Studies employing the mouse knockouts of *CACNA1F* do not faithfully recapitulate the human "synaptopathic" phenotype. Here we explore the potential of stem cell-derived human retinal organoids (HROs) to study synaptic defects in *CACNA1F*-mediated CSNB. ***Methodology:*** We generated *CACNA1F*-deleted iPSC lines from male WTC-11 iPSCs using the CRISPR/Cas9 system. HROs were generated from control and KO iPSC lines using the protocol established by our group. Control and knockout (KO) organoids were compared at day 100 (D100), D130, D160 and D190 by immunofluorescence analysis. *Results:* Control and KO organoids appeared externally similar and recapitulated the laminar arrangement of the photoreceptors and bipolar cells. However, KO HROs demonstrated a widened OPL (n=3 HROs, $P < 0.05$) with increased presynaptic Bassoon expression (n=3 HROs, $P < 0.05$) and bipolar cell neurite outgrowth (n=3). ***Conclusions:*** Our preliminary analysis suggests that the widened OPL in the KOs is due to supernumerary presynaptic complexes and ectopic branching of bipolar cell dendrites. Further analysis of these KO HROs for spatial distribution and apposition of pre- and post-synaptic proteins (Bassoon, Ribeye, mGluR6) and for neurite branching and arborization of bipolar cells ($GNG13^+$) will permit the characterization of synaptic miswiring in the disease state and provide a model for reversal of synaptic pathology. We expect that this study will allow us to delve further into mechanisms of synaptic assembly in human retina and lay the groundwork for future studies in HROs to model more complex aspects of retinal circuit development.

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Poster

603. Synapse Maturation and Remodeling

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

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Netherlands Organization for Scientific Research - Veni ZonMW 91618112 (HJB)
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Title: Scalable dynamics of synaptic growth in mammalian neocortex

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Abstract: The mammalian neocortex undergoes major age-related changes in synaptic density during postnatal life. In order to construct a comprehensive synthesis of the time course of this process, we analyzed a wide range of publications spanning over four decades in humans and other mammals. We extracted >16,000 data points from >130 studies, selected through the PRISMA systematic literature search pipeline, leveraging both conventional and natural language processing tools for screening to conduct the largest meta-analysis of neocortical synaptic maturation to date. Across nine species, synapse density followed a similar growth trajectory in which synapse density increased sharply after birth, reached peak density around one gestational period postnatal, and declined gradually over the next 4-5 gestational periods. This pattern persisted across neuronal subtype, synapse type and location, brain region, and neocortical layer. The exception was the human prefrontal cortex, whose peak synapse density was delayed to 10-15 gestational periods postnatal. We are now examining human neurodevelopmental disorders and corresponding animal models. Studies on idiopathic autism revealed prolonged post-peak elevation of synapse density compared with the neurotypical pattern, and idiopathic schizophrenia was associated with lower synapse densities in adulthood. Taken together, these findings provide a scalable framework for synaptic dynamics of neocortical development across the lifespan.

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Poster

603. Synapse Maturation and Remodeling

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

Support: NIGMS/NIH COBRE Grant no: P20GM121310-03
NIGMS Grant P20GM103432

Title: A role for serotonin in the maturation of a glutamatergic synapse

Authors: *U. G. UDOH[1], J. R. BRUNO[2], K. G. PRATT[1];
[1]Program in Neuroscience, Dept of Zoology and Physiol., [2]Dept of Zoology and Physiol., Univ. of Wyoming, Laramie, WY

Abstract: The *Xenopus laevis* tectum is the main visual processing center and the primary target for axons of retinal ganglion cells (RGCs), which project from the eye to form glutamatergic synapses onto tectal dendrites. The tectum is known to receive input from other brain areas, but little is known about central modulation of retinotectal transmission. Our recent finding that

serotonin (5-HT) modulates a visually guided behavior suggests that it may modulate the visual system. Here, we studied the modulating effect of 5-HT on retinotectal synaptic transmission in stage 48/49 tadpoles(10-18 days post-fertilization) by pharmacologically manipulating endogenous 5-HT levels followed by whole cell electrophysiological recording of RGC-evoked responses in tectal neurons. We found that increasing 5-HT transmission by 24 hours exposure of tadpoles to the selective 5-HT re-uptake inhibitor (SSRI) fluoxetine, caused a significant strengthening of maximum RGC-evoked responses. This effect was mediated by a combination of increase in probability of presynaptic transmitter release as well as increase in the strength of individual retinotectal synapses, a postsynaptic effect. Depleting serotonin by exposure of tadpoles to the tryptophan hydroxylase inhibitor p-chlorophenylalanine (p-CPA) caused a significant weakening of both the maximum RGC-evoked responses and the strength of individual synapses. To further investigate the mechanism of serotonin-induced strengthening of this glutamatergic synapse, we evaluated the effect of enhanced serotonergic transmission in the presence of NMDA receptor (NMDAR) blockade. We found that blocking NMDARs did not affect the fluoxetine-induced potentiation, indicating an NMDAR-independent strengthening. Alternatively, the synapses could be strengthened through phosphatidyl-inositol 3-kinase (PI3-K)-dependent signaling. Consistent with this, we found increased expression of the phosphoinositide PIP3(phosphatidylinositol-(3,4,5)-trisphosphate) in the soma of tectal neurons following exposure to fluoxetine, and inhibiting PIP3 synthesis using the PI3-K inhibitor LY294002 abolished the potentiating effect of fluoxetine on retinotectal transmission. Given that PIP3 has been shown to be important for postsynaptic AMPA receptor clustering and long-term potentiation, our data indicate that endogenous serotonin plays a role in the maturation of retinotectal synapses through PI3-K signaling.

Disclosures: **U.G. Udoh:** None. **J.R. Bruno:** None. **K.G. Pratt:** None.

Poster

603. Synapse Maturation and Remodeling

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Program #/Poster #: 603.09

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

Support: R15 NS108234-01

Title: The Role of Pathological Neuronal Activity in Oligodendrocyte Development, Neuronal-Glial Circuit Formation, and Myelination

Authors: I. TILTON[1], C. ARELLANO REYES[1], M. GARCIA[1], C. FRANZIA[1], H. NOGUCHI[2], R. BROCK[1], A. SAHAGUN[1], D. S. MOURA[2], S. J. PLEASURE[2], ***L. COCAS**[1];
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Abstract: Myelin sheaths, formed by oligodendrocyte cells in the CNS, are vital for rapid conduction of electrical signals down neuronal axons. In order for these myelin sheaths to form,

oligodendrocyte progenitors must differentiate and properly myelinate the axons during development and following demyelinating diseases. However, the mechanisms that drive the timing and specificity of this myelination is not well understood. Recent work has shown that oligodendrocyte progenitors receive synapses from neurons, providing a potential mechanism for neuronal-glial communication. We have previously shown that changing neuronal activity affects the proliferation of oligodendrocyte cells. We hypothesize that these neuron OPC connections and OPC differentiation will be altered during pathological neuronal activity. We used kainic acid to induce pathological neuronal activity, then analyzed changes in the rate of OPC proliferation and differentiation. We then combined viral monosynaptic circuit tracing along with immunohistochemical analyses of neuronal cell types to examine changes in circuit development after kainic acid-mediated epileptic activity. Next, we quantified changes in the expression of inhibitory and excitatory synaptic adhesion proteins on OPCs to analyze the effect of epileptic activity on the number and types of synapses onto OPCs. Finally, we measured changes in the myelination of neuronal axons to determine whether epileptic activity concomitantly affects neuronal glial connectivity and myelination rate. By using kainic acid as a model for pathological neuronal activity, we can better understand the importance of neuronal glial connections and the impact of epilepsy and other seizure disorders on OPC development and myelin formation, with the goal of understanding how these mechanisms may be recapitulated during remyelination after injury.

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Poster

603. Synapse Maturation and Remodeling

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Program #/Poster #: 603.10

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

Support: DBT- Government of India

Title: Store-operated calcium entry shapes developmental gene expression in dopaminergic neurons of the Drosophila flight circuit

Authors: *R. MITRA[1], S. RICHHARIYA[2], G. HASAN[1];
[1]NCBS, Natl. Ctr. for Biol. Sci., Bengaluru, India; [2]Hhmi/Brandeis Univ., Hhmi/Brandeis Univ., Waltham, MA

Abstract: Regulation of neuronal gene expression through activity-driven transcription of immediate-early genes has been well documented. Neuronal Ca^{2+} signals, with slower altered dynamics, occur upon stimulation of metabotropic receptors followed by IP_3-mediated intracellular Ca^{2+} release and store-operated Ca^{2+} entry (SOCE). A series of transcriptomic

screens in different neuronal contexts identified gene families whose expression is regulated by the IP_3R and key components of SOCE[1,2]. We have investigated transcriptional mechanisms by which IP_3/Ca^{2+} signaling and SOCE regulate the expression of genes in pupal and adult neurons in the context of *Drosophila* flight. In a recent study, we show that Set2, the Histone 3 lysine 36 methyltransferase forms part of a transcriptional feedback loop in a larval glutamatergic neuron subset to regulate the larval to pupal transition upon nutrient stress[3]. Here we investigate the role of Set2 in a subset of pupal and adult dopaminergic neurons required for maturation and function of the flight circuit. To bridge the gap between Ca^{2+} signals and expression of specific genes, including *Set2*, we used motif enrichment analysis across the upstream regions of genes targeted by SOCE and identified potential binding sites for the transcription factor GAF. We identify a critical pupal developmental window for flight circuit development wherein SOCE targets *Set2* expression through GAF activity and initiates deposition of the activating histone modifier H3K36me3 in a subset of dopaminergic neurons. This pathway is required for the timely expression of several ion-channel genes such as the voltage-gated Ca^{2+} channel subunit *cacophony* which regulates neuronal excitability and affects flight circuit output. In summary, we report a requirement for H3K36me3 downstream of GPCR signaling and SOCE in developing dopaminergic neurons which regulates the neuronal transcriptome, influences intrinsic excitability, and determines flight bout duration.
References1. Richhariya, S., Jayakumar, S., Abruzzi, K., Rosbash, M. and Hasan, G. (2017). A pupal transcriptomic screen identifies Ral as a target of store-operated calcium entry in Drosophila neurons. Sci. Rep. 7, 42582. Jayakumar, S., Richhariya, S., Deb, B. K. and Hasan, G. (2018). 2.Mitra R, Hasan G. (2022). Store-operated Ca^{2+} entry regulates neuronal gene expression and function. Curr Opin Neurobiol. 24;1025204. 3. Mitra R, Richhariya S, Jayakumar S, Notani D, Hasan G. (2021) IP_3-mediated Ca^{2+} signals regulate larval to pupal transition under nutrient stress through the H3K36 methyltransferase Set2. Development.148(11)

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Poster

603. Synapse Maturation and Remodeling

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

Support: NIH NINDS R21NS109641

Title: The Numb protein is required for growth, function, and plasticity at the Drosophila neuromuscular junction

Authors: T. SUKHANOVA[1], K. RENKEMEYER[2], N. PRITCHETT[2], B. A. BERKE[2], *H. KESHISHIAN[1];
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Abstract: Drosophila synaptic growth and plasticity at the NMJ depend in part on a TGF-beta growth factor provided by the muscle that regulates gene transcription at the motoneuron's cell body. When the growth factor is provided to only a subset of a motoneuron's synapses, normal growth and plasticity is restricted to those synapses alone. The growth factor both regulates gene expression at the nucleus and locally predisposes the growth factor-activated synapses to benefit from that expression. This is consistent with a "synaptic tagging and capture" model (Berke, B., Keshishian H., 2020. Dev. Neurobio.79:895–912). Here we examined the Numb protein as a candidate contributing to NMJ plasticity and as possibly involved in the local synaptic tagging we observe. Numb is asymmetrically located in the cytoplasm of precursor stem cells, regulating cell fate in both Drosophila and mammals. Numb is also located at presynaptic terminals of larval NMJs, as determined by immunolabeling and confirmed by reduced labeling after RNAi knockdown. The roles of Numb in NMJ development, function, and plasticity were tested by bipartite expression of UAS-Numb RNAi constructs with multiple GAL4 drivers ($Elav^{C155}$ GAL4 and the RU486-inducible Elav-GS-GAL4). Numb knockdown reduced NMJ synaptic contacts (boutons) by 19% when compared to parental controls ($Elav^{C155}$GAL4 driver; $p<0.0001$). Similarly, a 16% decrease was seen in RU486-induced vs uninduced larvae (Elav GS-GAL4 driving UAS Numb RNAi; $p<0.001$). Evoked junctional potentials were reduced, while spontaneous miniature potentials were unaffected, suggesting a significant reduction of quantal content (RU486-induced Elav-GS-GAL4 compared to uninduced controls). Finally, we asked if Numb function is involved in activity-dependent NMJ plasticity. Numb knockdown blocked the expected expansion of the NMJ following larval hyperactivity ($eag^{1}Sh^{120}$ K^{+} channel mutations). Numb RNAi knockdown in hyperactive $eag^{1}Sh^{120}$ motoneurons resulted in a 29% decrease ($p<0.003$) in boutons compared to hyperactive controls. The results indicate that Numb function is required for normal NMJ growth and plasticity. Given that Numb acts as an adapter for other membrane-targeted proteins, Numb may be well-suited to facilitate the tagging of NMJ synapses for plasticity.

Disclosures: T. Sukhanova: None. **K. Renkemeyer:** None. **N. Pritchett:** None. **B.A. Berke:** None. **H. Keshishian:** None.

Poster

603. Synapse Maturation and Remodeling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 603.12

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

Support: JP22bm08040005

Title: Quantitatively reproducible neuromuscular junction formation using human iPS cell-derived motor neurons and skeletal muscle cells

Authors: M. KAMON[1,2], S. SIEW[2], M. YAMASHITA[2,3], S. WAKATSUKI[2], ***T. ARAKI**[2,3];
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Abstract: Neuromuscular junction (NMJ) is a synapse that connects the nerve terminals of motor neuron (MN) with a skeletal muscle fiber. The mechanism of NMJ formation has been demonstrated mainly by studies in animal models. However, in order to elucidate the pathophysiological mechanisms and to search for therapeutic agents of human neuromuscular diseases, it is essential to set up a human NMJ model. Here, we established a human NMJ model utilizing a compartmentalized culture system with human pluripotent stem (iPS) cells-derived cells, which enables us to assess the NMJ formation quantitatively and reproducibly. We also confirmed the functionality of the formed NMJ using a fluorescent live cell imaging technique. Thus, our human NMJ model provides a powerful tool for elucidating pathological mechanisms and exploring therapeutic agents of human neuromuscular diseases.

Disclosures: M. Kamon: None. **S. Siew:** None. **M. Yamashita:** None. **S. Wakatsuki:** None. **T. Araki:** None.

Poster

603. Synapse Maturation and Remodeling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 603.13

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

Support: NIH R01 HD092369

Title: Maternal Exercise-Regulated Irisin and Leptin Enhance Synaptogenesis in the Developing Hippocampus

Authors: *M. JOSTEN[1], K. PARKER[1], C. KELLEY[2], J. RODRIGUEZ LLAMAS[3], M. ZHU[2], G. WAYMAN[1];
[1]Washington State Univ. Grad. IPN, Washington State Univ. Grad. IPN, Pullman, WA;
[3]Washington State Univ. Grad. IPN, [2]Washington State Univ., Pullman, WA

Abstract: Hippocampal (HPC)-linked neurodevelopmental disorders including autism spectrum disorder and schizophrenia have increased in recent years. Their etiology is multifaceted but gestational environment and the intrauterine hormonal milieu is thought to contribute to their development. In this context, it is known that acute and chronic exercise during pregnancy exert neuroprotective effects on HPC-dependent learning and memory in offspring. Specifically, irisin, a peptide produced by cleavage of Fibronectin type III domain-containing protein 5, is upregulated and released during exercise and works to promote adult HPC neurogenesis and synaptogenesis. Leptin, another procognitive neuropeptide, is critical for HPC synaptic development, and leptin sensitivity improves with exercise. The expression and activity of these peptides can be modulated by exercise, but how maternal exercise (ME) impacts their regulation

in the developing HPC has not been investigated. Using immunohistochemistry, confocal microscopy, biochemistry, and electrophysiology, we studied *in vitro* models of developing rat HPC neurons to identify interacting leptin- and irisin-activated signaling pathways. We performed *in vivo* C57BJ mouse studies to determine the effects of daily ME on HPC gene expression and glutamatergic synaptogenesis. HPC morphology and mRNA expression was compared between the offspring of maternal treadmill exercise (n = 3 adult females) or maternal unexercised control (n = 2 adult females) mice. The *in vitro* data revealed that irisin and leptin stimulation of HPC neurons increase the number of functional glutamatergic synapses (mature dendritic spines: One-Way ANOVA Tukey $p<0.05$; mini excitatory post-synaptic current frequency: One-Way ANOVA Tukey $p<0.019$) in a co-dependent, brain-derived neurotrophic factor-dependent manner compared to non-stimulated control neurons. We also found that irisin increases ERK activation in developing neurons through an integrin-dependent mechanism. Our *in vivo* studies reveal that ME promotes increased glutamatergic synaptogenesis in neonatal mouse pups compared to the offspring of sedentary mothers, corroborating our *in vitro* studies. While the connection between exercise and HPC synaptogenesis is well known, the molecular mechanisms by which different maternal physiological states contribute to HPC development are not as well understood. Together, these data suggest that irisin is a link between ME and offspring HPC function. Further elucidation of this mechanism may inform new routes for therapeutic interventions against neurodevelopmental disease.

Disclosures: M. Josten: None. **K. Parker:** None. **C. Kelley:** None. **J. Rodriguez Llamas:** None. **M. Zhu:** None. **G. Wayman:** None.

Poster

603. Synapse Maturation and Remodeling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 603.14

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

Support: NIH Grant HD092369

Title: Brain-derived neurotrophic factor signaling is required for the neurotrophic effects of leptin at glutamatergic synapses in the developing hippocampus

Authors: *J. RODRIGUEZ LLAMAS, C. DILLON, G. A. WAYMAN;
Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: Neurotrophic factors direct the development of the nervous system, and impairments in their function lead to neurological disorders. The adipokine leptin, which is involved in energy balance, exerts neurotrophic effects in the central nervous system. Leptin is also synthesized in the hippocampus, promoting spinogenesis, synaptic plasticity, long-term potentiation, and neurogenesis, while rodent models with altered leptin signaling exhibit impairments in these processes and defects in hippocampal-related functions, such as impaired learning and spatial

memory. Notably, altered leptin signaling is also associated with psychiatric and cognitive disorders in humans, such as depression and Alzheimer's disease. Leptin increases the number of dendritic spines and the frequency of mini excitatory postsynaptic currents (mEPSC) in hippocampal pyramidal neurons, evidencing an increase in the number of functional glutamatergic synapses. Given the putative role of dendritic spines on learning and memory, leptin's role in spinogenesis may explain the defects observed in rodent models with leptin-deficient signal. Nonetheless, the molecular mechanisms underlying leptin neurotrophic effects are poorly understood. Interestingly, leptin increases mRNA and protein expression of brain-derived neurotrophic factor (BDNF) in adult mice, suggesting that BDNF could work downstream of leptin signaling. Using a combination of cell and molecular biology, biochemistry, fluorescence, and microscopy approaches, we investigated both *in vitro* and *in vivo* the role of BDNF and its receptor Tropomyosin receptor kinase B (TrkB) for the neurotrophic effects of leptin in the developing hippocampus. We observed that the BDNF-TrkB signaling is required for leptin effects in the hippocampus both *in vitro* and *in vivo*, as inhibition of BDNF release or TrkB activation, as well as knockdown of BDNF or TrkB, prevented leptin effects on dendritic spines. We also investigated the molecular mechanisms underlying this requirement, and we observed that leptin not only increases BDNF mRNA expression but also promotes BDNF secretion. Specifically, we found that leptin-dependent BDNF secretion required transient receptor potential C (TrpC) 1 and 3 channels, as well as Ca^{2+} release from intracellular stores and Ca^{2+} influx through voltage-gated calcium channels (VGCCs). Thus, our results reveal a new regulatory effect of leptin on BDNF secretion and suggest that BDNF-TrkB signaling is necessary for leptin effects on the structural plasticity of dendritic spines in the pyramidal neurons of the developing hippocampus.

Disclosures: J. Rodriguez Llamas: None. **C. Dillon:** None. **G.A. Wayman:** None.

Poster

603. Synapse Maturation and Remodeling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 603.15

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

Title: Zinc causes toxicity and impairs network activity in human cortical neurons

Authors: *R. HODGSON[1], J. E. HADDON[2], M. ALSAQATI[2], D. CABEZAS[2], Y. ZHU[2], S. WAINWRIGHT[2], P. R. MAYCOX[3], M. PAPAKOSTA[4], J. SCHACHTER[5], D. L. BUHL[6], M. LI[2], L. GRAY[2], A. HARWOOD[2], J. HALL[2], L. WILKINSON[2];
[1]Takeda, Takeda, San Diego, CA; [2]Cardiff Univ., Cardiff, United Kingdom; [3]Discovery Biol., Takeda Cambridge, Cambridge, United Kingdom; [4]Takeda Develop. Ctr. Americas, Inc., San Diego, CA; [5]Neurosci., Takeda Pharmaceut. Co., San Diego, CA; [6]Neurosci. Res. Unit, Sage Therapeut., Cambridge, MA

Abstract: Motivated by evidence from genetics for association between ZIP8 (SLC39A8), a transporter protein mediating influx of Zn^{2+} into cells we investigated the effects of Zn^{2+} in human cortical neurons. Zn^{2+} plays a key role in multiple cellular processes and is required for normal neuronal development and function. Work in rodent models has shown that synaptic Zn^{2+} modulates the function of neurotransmitter receptors, inhibiting N-methyl-D-aspartate (NMDA), g-aminobutyric acid (GABA) receptors and transporter-mediated glutamate uptake, whilst having a concentration-dependent impact on a-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid receptor (AMPAR) function. Additionally, work in animal models has shown that excess Zn^{2+} results in neurotoxicity. Here we report the impact of Zn^{2+} on human brain tissue utilizing stem cell-derived cortical neurons. Neurons plated onto a multi-electrode array (MEA) enabled monitoring of the effects of manipulating extracellular Zn^{2+} on electrophysiological activity. Initially we used concentrations of extracellular Zn^{2+} (5-10uM $ZnCl_2$) used typically in previous rodent work. However, when applied to the human neuron model, these concentrations of Zn^{2+} resulted in a profound loss of electrophysiological activity such that the pre-manipulation synchronous network activity of the neurons was abolished and the activity of individual neurons was markedly reduced. These effects were irreversible following the return to baseline levels of Zn^{2+}. Post-study cultures examined using microscopy demonstrated that Zn^{2+}exposure at these concentrations resulted in time-dependent morphological changes in the neurons, beginning with swelling of neuronal somata within the initial 10min of exposure and cytoplasmic granularity and disintegration observed after 30min, resulting in neuronal death. In contrast, exposure to the same concentrations (and higher, up to 20uM $ZnCl_2$) were not toxic to human stem cell cultures. Further work employing a lower concentration of extracellular Zn^{2+} exposure (200nm $ZnCl_2$) did not induce cell death in the neurons but did result in disrupted synchronisation of network neuronal activity. At this lower concentration, normal neuronal activity was restored following washout. These data reveal the particular sensitivity of human neurons to extracellular Zn^{2+} and suggest that perturbations of Zn^{2+} homeostasis may underlie pathological conditions contributing to neurodegeneration and abnormal synaptic function in brain disorders such as schizophrenia. Future studies will include genetic manipulation of ZIP8 relevant to CNS disorders.

Disclosures: **R. Hodgson:** A. Employment/Salary (full or part-time):; Takeda. **J.E. Haddon:** None. **M. Alsaqati:** None. **D. Cabezas:** None. **Y. Zhu:** None. **S. Wainwright:** None. **P.R. Maycox:** A. Employment/Salary (full or part-time):; Takeda. **M. Papakosta:** A. Employment/Salary (full or part-time):; Takeda. **J. Schachter:** A. Employment/Salary (full or part-time):; Takeda. **D.L. Buhl:** None. **M. Li:** None. **L. Gray:** None. **A. Harwood:** None. **J. Hall:** None. **L. Wilkinson:** None.

Poster

603. Synapse Maturation and Remodeling

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 603.16

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

Support: NIH Grant R01NS065856
NIH Grant F3113577899
NIH Grant R01NS105628
NIH Grant R01NS102937

Title: A gene therapy approach for treating pharmacoresistant epilepsy using Sema4D

Authors: *S. ADEL[1], A. EVANS-STRONG[2], V. CLARKE[3], J. L. MAGUIRE[5], S. PARADIS[4]; [1]Brandeis Univ. Grad. Neurosci. Program, Waltham, MA; [2]Neurosci., Tufts Univ., Boston, MA; [4]Biol., [3]Brandeis Univ., Waltham, MA; [5]Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Status epilepticus (SE) is a life-threatening neurological emergency characterized by continuous seizure activity lasting greater than 5 minutes. First line treatment for SE is intravenous or intramuscular administration of benzodiazepines (BZD; e.g. diazepam), which enhances the activity of $GABA_A$ receptor subunits and counteracts excess neuronal activity. However, the need for innovative anti-seizure medications to treat SE is dire because ~30% of SE patients do not respond to treatment with BZD plus at least one other anti-seizure medication, resulting in refractory SE (RSE). We previously demonstrated that intra-hippocampal infusion of purified, Semaphorin 4D (Sema4D) extracellular domain into the mouse hippocampus rapidly promotes formation of GABAergic synapses and decreases seizure susceptibility in mice. Here, we created an Adeno Associated Virus expressing the extracellular domain of Sema4D (AAV-Sema4D-ECD) and demonstrate its ability to promote GABAergic synapse formation in organotypic slice cultures. We further show that direct hippocampal injection of AAV-Sema4D-ECD reduced seizure severity and restored sensitivity to diazepam treatment in an *in vivo* kainic acid model of SE. Thus, we provide proof of concept that viral delivery of Sema4D can promote GABAergic synapse development and can combat benzodiazepine insensitivity in intractable forms of epilepsy, paving the way for efficacious therapies in the future.

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Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.01

Topic: A.07. Developmental Disorders

Support: 2018R1D1A1B07043238
IBS-R002-A1
2018H1A2A1059772

Title: Requirement of AGO1 for sociability and structuring the brain architecture

Authors: *H. DO[1], D. KIM[1], M. JU[1], R. JAPPELLI[3], H. YANG[2], Y. JEON[2], G. SON[1], J. SON[1], I. AHN[1], C. LIM[3], Y. JU[2], Y.-M. HAN[2], S.-H. LEE[2], D. LEE[2], F. H. GAGE[3], J. HAN[1]; [1]Grad. Sch. of Med. Sci. and Engin., [2]Biol. Sci., Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of; [3]LOG-G, Salk Inst., La Jolla, CA

Abstract: Diverse de novo variations on the *AGO1* gene have been identified in human patients with neurodevelopmental disorders, including intellectual disability and autism spectrum disorders (ASD). While molecular functions of AGO1 in RNA interference are very well understood, the biological roles of AGO1 in brain development and diseases remain largely unknown. Here, we present the requirement of AGO1 for sociability and structuring brain architecture during early development. First, we confirmed that AGO1 depletion in neurons results in behavioral changes in animals, similar to patients carrying AGO1 variants. Ago1 knockout (KO) in neurons leads to hyposociability of animals, which is one of the representative phenotypes of ASD. Next, we produced AGO1 KO human embryonic stem cells by applying the CRISPR/Cas9 technique and differentiated them into forebrain organoids, which mimic the 3D structure of the developing brain. Ablating AGO1 remarkably disrupts the ventricle-like structure of human forebrain organoids. Approximately half of the ventricle-like structures are unclosed, and the ventricular zone-like structures are significantly thicker in AGO1 KO organoids. At the cellular level, AGO1 KO leads to a change in neuronal morphology and electrophysiological property. AGO1 KO neurons contain shorter neurites and fire less compared to the controls. Supporting the phenotypes, differentially expressed genes (DEGs) in AGO1 KO neural progenitor cells (NPCs) and neurons were related to the extracellular matrix, cell adhesions, and neuronal differentiation. Among the DEGs, RELN, a key signaling molecule for neuronal migration and development, decreased with the most remarkable fold change in NPCs. By performing chromatin immunoprecipitation, we found that AGO1 binds to the chromatin. One of the regions associated with AGO1 in NPCs is the promoter of LIN28A that regulates the translation of mRNAs destined for the endoplasmic reticulum. We are currently working on untangling gene networks related to the phenotypes observed in AGO1 KO neurons. Nevertheless, our data clearly show that AGO1 is essential in the early stages of brain development, forming a ventricle-like structure.

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Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.02

Topic: A.07. Developmental Disorders

Support: R21MH118685
NSF PRFB 2011039

Title: Murine global genetic diversity captures trait heterogeneity in penetrance of Chd8 haploinsufficiency

Authors: *M. TABBAA, A. KNOLL, P. LEVITT;
Children's Hosp. Los Angeles, Los Angeles, CA

Abstract: Pre-clinical models of neurodevelopmental disorders (NDD) typically use single inbred strains which fail to capture symptom heterogeneity that is common clinically. We tested if systematically modeling human genetic diversity in mouse genetic reference panels recapitulates population and individual differences to a syndromic mutation in the high-confidence autism risk gene, *Chd8*. B6-*Chd8* heterozygous (*Chd8*$^{+/-}$) dams were mated with sires from 27 collaborative cross, 5 BXD and B6 strains to produce F1 B6-CC, B6-BXD and B6-B6 male and female wild-type (WT) or *Chd8*$^{+/-}$ littermates. Body weight trajectories, adult social behaviors, contextual fear learning, anxiety-like and ambulatory behaviors and finally, brain weights (proxy for brain size) were measured. Integrative and comparative analyses determined unique and shared variance of the impact of *Chd8* haploinsufficiency across traits within and across the genetically diverse set of GRP strains. Cohen's D effect size estimates reported for *Chd8* population and strain effects provide key contexts of significant findings that allow direct comparison of effect magnitudes. While trait disruption mimicked those in humans at a population level, including high penetrance of macrocephaly and disrupted behavior, there were robust strain and sex differences. For every trait, a subset of individual strains exhibited a range of large effect sizes, sometimes in opposite directions, and remarkably others expressed resilience. There were significant contributions of sex to the impact of *Chd8* haploinsufficiency across traits. Altogether, these data provide a powerful framework for broad application to discovery of NDD etiologies and translation for treatment discovery that is best aligned with heterogeneity of clinical symptoms.

Disclosures: M. Tabbaa: None. **A. Knoll:** None. **P. Levitt:** None.

Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.03

Topic: A.07. Developmental Disorders

Title: A novel murine model to assess the Wac gene, the cause of DeSanto-Shinawi syndrome and high risk autism associated gene

Authors: A. M. STAFFORD[1], M. PACHECO-VERGARA[2], J. JEONG[2], *D. VOGT[1];
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Abstract: Several monogenic syndromes are associated with neurodevelopmental changes that result in cognitive impairments, including autism, attention deficit hyperactivity disorder (ADHD) and seizures. Limited studies and resources are available to make meaningful headway into the underlying mechanisms that result in these symptoms. One such example, DeSanto-Shinawi Syndrome (DESSH), is a rare disorder caused by mutations in the *WAC* gene. Those diagnosed with DESSH experience craniofacial alterations as well as cognitive symptoms that include autism, ADHD and seizures. While some data exists examining *WAC*, studies in vertebrate brains is lacking. To overcome this, we generated constitutive murine *Wac* mutants and assessed phenotypes that are relevant to humans diagnosed with DESSH. *Wac* mutants have craniofacial, anatomical, behavioral and seizure susceptibility that are relevant to DESSH; this new model is suited to study some of the core symptoms of DESSH and the biology of *Wac*. Moreover, this model makes headway into the arsenal of genetic tools needed to assess the neurological changes that may be common in autism, ADHD and that underlie seizures in this and other disorders.

Disclosures: A.M. Stafford: None. **M. Pacheco-Vergara:** None. **J. Jeong:** None. **D. Vogt:** None.

Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.04

Topic: A.07. Developmental Disorders

Support: NIH Grant NS116089

Title: Novel ASTN2 mutant mouse shows aberrant cerebellar synaptic structure and physiology and exhibits ASD like behaviors

Authors: *M. HANZEL[1], K. FERNANDO[2], S. GONG[3], S. E. MALONEY[4], J. D. DOUGHERTY[4], C. HULL[2], M. E. HATTEN[1];
[1]Rockefeller Univ., New York, NY; [2]Neurobiolgy Dept, Duke Univ., Durham, NC; [3]Weill Cornell Med., New York, NY; [4]Psychiatry, Washington Univ. Med. Sch., Saint Louis, MO

Abstract: The cerebellum has essential roles in non-motor behaviors and cognition and contributes to neurodevelopmental disorders such as autism spectrum disorder (ASD). Here, we examine the role of the predominantly cerebellar gene ASTN2 in cerebellar circuit function and ASD-related behaviors. Copy number variations in ASTN2 have been identified as a significant risk factor for ASD. Our previously published cellular and molecular studies show that ASTN2 binds to and regulates the trafficking of multiple synaptic proteins, including neuroligins, which have been genetically linked to ASDs, and modulates cerebellar Purkinje cell (PC) synaptic activity. To provide a genetic model to study cerebellar circuit function and the role of the cerebellum in neurodevelopmental disorders, we generated a global *Astn2* knockout (KO) mouse

line. Our electrophysiological experiments indicate a reduced frequency of spontaneous EPSCs, as well as increased amplitudes of both spontaneous EPSCs and IPSCs in the KO animals, suggesting that pre- and post-synaptic components of synaptic transmission are altered in the *Astn2* KO. We also found a pronounced reduction in PC dendritic spine density using Golgi staining, and, with immunohistochemistry, a reduction of excitatory synapses between granule cells parallel fibers and PCs, and a reduction in inhibitory synapses between PCs and interneurons. These results suggest a specific cerebellar circuit defect in the *Astn2* mutants. In addition, *Astn2* KO mice exhibit strong ASD-related behavioral phenotypes. *Astn2* KO pups show a marked decrease in the number of calls in the separation-induced pup ultrasonic vocalizations test as well as characteristics of less complex calls. The juvenile *Astn2* KO mice show hyperactivity, anxiety, and repetitive behavior phenotypes in the open field test. Additionally, *Astn2* KO mice exhibit altered social behavior in the three-chamber test, showing both reduced sociability as well as lower social novelty preference. In proteomic experiments, we found that *Astn2* KOs show marked upregulation of its family member, ASTN1, a protein well characterized for its role in glial-guided neuronal migration in the cerebellum. Consequently, using electron microscopy and immunohistochemistry we find a large increase in Bergmann glia volume in the molecular layer of *Astn2* KO animals. We propose that the *Astn2* KO mouse is an exciting novel cerebellar-based mouse model that allows for a detailed study of the interaction between the cerebellum, glia, and ASD.

Disclosures: M. Hanzel: None. **K. Fernando:** None. **S. Gong:** None. **S.E. Maloney:** None. **J.D. Dougherty:** None. **C. Hull:** None. **M.E. Hatten:** None.

Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.05

Topic: A.07. Developmental Disorders

Support: PACS1 Foundation

Title: Neural precursor cell models of PACS1 syndrome have impaired migration dynamics

Authors: *E. G. POPE, L. E. RYLAARSDAM, A. GUEMEZ-GAMBOA;
Neurosci., Feinberg Sch. of Med., Chicago, IL

Abstract: Intellectual disability (ID) is a highly prevalent neurodevelopmental disorder affecting roughly 1% of the U.S. population. Though thousands of genetic variants contribute to ID, in rare cases the etiology can be traced to a single pathogenic variant, providing an opportunity to investigate disease mechanisms in a more constrained model. PACS1 syndrome is an ID caused by a single *de novo* p.R203W substitution in phosphofurin acidic cluster sorting protein 1 (PACS1). Previous research on PACS1 has revealed roles in both the endosomal pathway and the nucleus, where it binds to partners including histone deacetylases. Previous single-cell RNA

sequencing meta-analyses from the lab showed an upregulation of genes involved in neuron migration in PACS1$^{(+/R203W)}$ cells, which could result in improper circuit formation and lead to ID. Despite our knowledge of the symptoms of PACS1 syndrome and some of its effects on the developing brain, it remains unknown if this mutation is a loss-of-function, dominant-negative, or gain-of-function. In line with this, previous research has shown that knocking out the PACS1 protein in mice had no effect on the nervous system and a family with a multi-exon deletion of the PACS1 protein did not present with any of the typical symptoms of PACS1 syndrome. Given this observation and previous research showing differential migration of neural crest cells upon overexpression of p.R203W PACS1, we hypothesized PACS1$^{(+/R203W)}$ neural cells would have migration defects and PACS1-KO neural cells would have the same migration as control cells. To test this hypothesis, we grew neural precursor cells (NPCs) derived from both patient and control iPSCs into neurospheres and we found that PACS1$^{(+/R203W)}$ NPCs migrate further than controls. Furthermore, knocking out the PACS1 protein resulted in a different migration phenotype than that found in PACS1$^{(+/R203W)}$ NPCs. Our results also demonstrate that PACS1-KO NPCs have the same rate of division and apoptosis as control NPCs, suggesting that knocking out the protein is a promising option to eliminate the potential gain-of-function mutation. RNA Sequencing revealed that PACS1 KO and PACS1$^{(+/R203W)}$ are associated with different transcriptomic changes. Knockout of the PACS1 protein may be used in the future not only as a treatment for PACS1 syndrome. Mechanisms revealed from this research may also be used to treat ID from diverse genetic etiologies.

Disclosures: E.G. Pope: None. **L.E. Rylaarsdam:** None. **A. Guemez-Gamboa:** None.

Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.06

Topic: A.07. Developmental Disorders

Support: Departmental grant AIMS-2-TRIALS

Title: Aggression in mouse models of Autism Spectrum Disorder (ASD)

Authors: *A. A. HERTZ, G. MCALONAN, N. BLACKWOOD, M. M. PETRINOVIC; Dept. of Forensic and Neurodevelopmental Sci., Inst. of Psychiatry, Psychology, and Neuroscience, King's Col. London, London, United Kingdom

Abstract: Aggression is a common form of challenging behavior among individuals with autism spectrum disorder (ASD). Affecting as many as 2-out-of-3 individuals with ASD, aggression has serious detrimental effects. Aggression often exacerbates core ASD symptoms and is the strongest predictor of parental stress, yet we lack effective treatments. Thus, a neurobiologically informed approach to the understanding and treatment of aggression in ASD is needed.
In this project we aimed to investigate the neurobiological correlates of ASD-associated

aggression using the *Neurexin1α* (*Nrxn1α*) knock-out (KO) and *Neuroligin-3* (*Nlgn3*) knock-in (KI) mouse models. Mutations in these two genes have been associated with aggression in both humans and animal models of ASD. Male *Nrxn1α* KO and *Nlgn3* KI mice were first tested for sociability and aggression with either male or female intruders in a resident/intruder test, and then ex-vivo anti-c-Fos immunohistochemistry was performed to investigate the neural activity changes associated with aggression.
Our results show that *Nrxn1α* KO and *Nlgn3* KI mice exhibit increased aggression compared to wild-type mice. C-Fos immunohistochemistry revealed increased activity of both the aggression- and reward-related brain circuits - suggesting that aggressive behavior of these mice may be associated with an aberrant functioning of the reward circuitry. Thus, our study suggests the reward system as a potential drug target for the treatment of ASD-associated aggression.

Disclosures: A.A. Hertz: None. **G. McAlonan:** None. **N. Blackwood:** None. **M.M. Petrinovic:** None.

Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.07

Topic: A.07. Developmental Disorders

Support: SLC6A1 connect
Simons Foundation Autism Research Initiative: SFARI

Title: Gene Therapy for SLC6A1 neurodevelopmental disorders improves behavioral and molecular disease phenotypes

Authors: *S. CHORNYY, N. PYNE, C. HOLAWAY, J. REDIGER, F. S. ROUSSEL, A. SUNSHINE, A. SIERRA DELGADO, S. LIKHITE, K. MEYER, A. BRADBURY;
Gene Therapy, Nationwide Children's Hosp., Columbus, OH

Abstract: The electrogenic sodium and chloride-coupled γ-aminobutyric acid (GABA) transporter GAT-1 is one of the main transporters in the central nervous system responsible for the reuptake of the inhibitory neurotransmitter GABA from the synapse. Consequently, a mutation in one copy of the SLC6A1 gene encoding GAT-1 results in loss of its function and leads to neurodevelopmental disorder including epileptic encephalopathy and autism spectrum disorder. Gene replacement strategies represent a viable therapeutic approach to treating patients with SLC6A1 mutations. However, the success of the gene therapy approach depends on efficient cellular targeting of disease-relevant cell types including inhibitory and GABAergic neurons, as well as astrocytes. Adeno-associated virus (AAV) gene therapy presents one of the most potent solutions for human neurological diseases with a genetic etiology. We leveraged the natural neuronal and astrocytic tropism of the AAV serotype 9 (AAV9) capsid and further regulated expression level and cell type specific transduction by using different promoters to

drive transgene expression. We used a novel mouse model mimicking a patient mutation (S295L) for efficacy testing. Neonatal wild type and S295L mice were injected intracerebroventricularly with AAV9 constructs carrying various promoters in a dose-dependent manner, which helped us identify cell-specific targeting, tissue-dependent biodistribution, and the most effective dose without toxic effects. Our study demonstrated that S295L mice treated with our lead candidate vector had substantial improvement in behavioral tests, such as cage hanging and elevated plus maze, and displayed reduced number of seizures. In addition, this cohort of treated animals showed normalized weight gain and correction of the clasping defect, which were very pronounced in untreated S295L mice. Importantly, the treatment was safe and well tolerated in wild type mice. This comparison study allowed us to identify the most suitable gene therapy construct and dose for further development towards clinical translation.

Disclosures: S. Chornyy: None. **N. Pyne:** None. **C. Holaway:** None. **J. Rediger:** None. **F.S. Roussel:** None. **A. Sunshine:** None. **A. Sierra Delgado:** None. **S. Likhite:** None. **K. Meyer:** 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.; Alcyone Therapeutics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Alcyone Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alcyone Therapeutics. F. Consulting Fees (e.g., advisory boards); Alcyone Therapeutics. **A. Bradbury:** None.

Poster

604. Autism: Models

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Program #/Poster #: 604.08

Topic: A.07. Developmental Disorders

Support: European Union's Horizon 2020 Research and Innovation Programme (Marie Sklodowska-Curie Global Fellowship, No. 845065).

Title: Mapping the neuroconnectional landscape in autism via cross-species fMRI

Authors: *M. PAGANI[1,2], V. ZERBI[3], A. GALBUSERA[1], T. XU[2], N. WENDEROTH[3], M. MILHAM[2], A. DI MARTINO[2], A. GOZZI[1];
[1]Inst. Italiano Di Tecnologia, Genova, Italy; [2]Child Mind Inst., New York City, NY; [3]Neural Control of Movement Lab, ETH Zurich, Zurich, Switzerland

Abstract: Background. Autism is characterized by high etiological heterogeneity. Brain imaging studies have revealed atypical functional connectivity in individuals with autism as measured by resting-state fMRI (rsfMRI). In keeping with the large etiological and biological variability of the disorder, diverse and diverging patterns of connectivity alterations have been reported in individuals with autism. However, the significance of heterogeneity in rsfMRI

connectivity remains largely debated. By back-translating rsfMRI mapping in physiologically accessible species like the mouse, we recently showed the possibility to reliably map autism-related connectivity alterations with exquisite etiological specificity. Here we leveraged our mouse database to define a “ground-truth” etiologically-relevant neuroconnectional landscape of autism, and to inform analogous efforts in relevant clinical populations. **Methods**. ***Mice:*** A total of 286 mice with 19 autism-related genetic alterations and 290 control littermates underwent rsfMRI mapping at 7T. Twelve etiologies were scanned at IIT Rovereto (Italy) and 7 etiologies were scanned at ETH Zürich (Switzerland). RsfMRI connectivity mapping was carried out by using global connectivity mapping. ***Humans:*** Global connectivity mapping was carried out on n=1123 individuals with ASD and n=1166 controls (6-30 yo) from ABIDE1 (Di Martino et al., 2014) and ABIDE2 (Di Martino et al., 2017). Replicability of findings was assessed by splitting our sample into discovery and replication datasets. **Results**. Consistent with our recent work (Zerbi et al., 2020), we found that most etiologies had robust connectivity alterations, and many exhibited distinctive functional disconnections. To assess whether this variability can be parsed in functionally-meaningful subtypes, we used hierarchical cluster analysis. Results revealed at least two subtypes showing diverging patterns of connectopathy. We next turned to human rsfMRI to obtain an analogous assessment of the connectional landscape. Here we found that rsfMRI connectivity mapping in subjects with idiopathic autism defined a pseudo-continuous landscape of connectivity alterations remarkably similar to the one mapped in the mouse database. Notably, we found an analogous landscape of autism-related atypicalities in the replication dataset. **Conclusion.** Our cross-species approach suggests that atypical functional connectivity in autism can be conceptualized by a pseudo-continuous neuroconnectional landscape. Our findings support the notion that there is not unique signature of connectopathy for the disorder.

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Poster

604. Autism: Models

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Topic: A.07. Developmental Disorders

Support: NRF-2022R1A6A3A13073152
HI18C10770300

Title: Effects of CLCN4 variants on human neurodevelopmental process

Authors: *D. KIM[1], H. DO[1], C. KANG[1], I. AHN[1], Y. KOH[1], J. KIM[3], G. SON[1], J.-H. SON[1], M. JU[1], D. KIM[3], J.-E. PARK[1], J. HAN[2,1];
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Abstract: Variations in different positions along the *CLCN4* have been identified in patients with intellectual disability, autistic traits, and epileptic seizures. *CLCN4* is a gene encoding voltage-gated chloride channel 4, CLC-4. The expression profile of the *CLCN4* shows the highest expression level in the brain among the other tissues, and patients carrying *CLCN4* variants present symptoms related to brain dysfunction. However, the biological roles and characteristics of CLC-4 in brain development and diseases remain largely unknown. In this study, to investigate the effects of *CLCN4* variations on the neurodevelopmental process, we generated human embryonic stem cells (hESCs) carrying *CLCN4* variants by applying the CRISPR/Cas9-based genome editing technique. At first, we differentiated the hESCs carrying the *CLCN4* variant to neural progenitor cells (NPCs) and obtained adequate NPCs similar to the control. Next, NPCs were differentiated into neurons. The neurons with the *CLCN4* variant displayed abnormal morphology of extended neurites at the early stage of neurogenesis. Surprisingly, the neurons with *CLCN4* variants suddenly died before maturation. Even in the forebrain organoids, where non-neuronal cells in the structure can support neuronal development, TUJ1 positive cells were drastically reduced if *CLCN4* carries genetic variations. The single-cell RNA sequencing results indicate that the brain organoids with *CLCN4* variants contain reduced number of neurons at the early time point in the pseudo-time-based trajectory. In summary, our results show that *CLCN4* is essential for the neuronal viability at the early stage of neurodevelopment. Furthermore, in this presentation, we will also discuss the expected molecular mechanisms underlying the cell death of neurons with *CLCN4* variants that we are currently investigating.

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Poster

604. Autism: Models

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Program #/Poster #: 604.10

Topic: A.07. Developmental Disorders

Support: ZIAAA000440

Title: Examining the brain-wide expression of dopamine $D1^+$ $D2^+$ receptor co-expressing neurons

Authors: *J. ABRAMOVITZ, M. W. ANTOINE, Y. SHEN;
Section on Neural Circuits, NIAAA/NIH, Rockville, MD

Abstract: Dopamine (DA) signaling drives motor behavior that, when impaired, manifests motor deficits. DA signaling is mediated by two often dissociable populations of neurons expressing either D1 or D2 receptors (D1R, D2R). Within the striatum, D1R cells participate in

the direct pathway which initiates goal-directed actions, while D2R cells participate in the indirect pathway to oppose movement in part by regulating the function of D1R cells. Although the expression and function of these segregated populations is well-characterized, less is known about a subset of cells that co-express D1R and D2R. These D1R/D2R-co-expressing cells have recently been shown to be expressed in greater numbers in the subpallidum of mice with 16p11.2 deletion, a genetic mouse model of autism spectrum disorder (ASD). Interestingly, 16p11.2 mice are hyperactive and display motor impairments, suggesting a link between motor dysfunction and elevations in D1R/D2R co-expression. In this study, we investigated the functional role of D1R/D2R co-expressing cells to better understand the underlying etiology of the ASD model. We first used a double transgenic reporter line (*Drd1-tdTmto;Drd2-eGFP)* combined with light sheet imaging to quantify brain-wide expression of D1R-, D2R-, and D1R/D2R-co-expressing populations in wildtype and 16p11.2 brains. These data showed an enrichment of D1R/D2R co-expressing cells in cortical layer 2/3 specifically in areas that overlap the default mode network (DMN), a group of midline areas thought to underly theory of mind, a cognitive attribute impaired in ASD patients. Furthermore, 16p11.2 brains exhibited an increased number of D1R/D2R-co-expressing neurons in the retrosplenial cortex, critical to the mouse DMN. We next investigated the functional output of these cells by selectively chemogenetically inhibiting D1R/D2R co-expressing cells and measuring effects on D1R mediated locomotion. Reduced output from D1R/D2R co-expressing neurons resulted in reduced modulation of striatal DA pathways and abnormal locomotor activity levels. Taken together, these data suggest that altering the activity in D1R/D2R co-expressing neuron can impair motor function and contribute an additional level of modulation on motor control. We are also expanding our analyses beyond the striatum to investigate what role this population plays in the cortex, particularly the DMN. This research will refine our understanding of roles these cells play in DA signaling as well as how their expression is affected by genetic conditions relevant to ASD.

Disclosures: **J. Abramovitz:** None. **M.W. Antoine:** None. **Y. Shen:** None.

Poster

604. Autism: Models

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Program #/Poster #: 604.11

Topic: A.07. Developmental Disorders

Support: NIH R01 MH094527
Brain Foundation Award
NIH F32 MH120966
NARSAD YI Award

Title: Expression of the hypermorphic SERT Gly56 Ala substitution induces tonic innate immune activation in the CNS in vivo

Authors: *A. E. WALSH[1], R. M. KATAMISH[1], P. A. GAJEWSKI-KURDZIEL[1], M. J. ROBSON[2], K. C. O'REILLY[3], J. VEENSTRA-VANDERWEELE[3], N. QUAN[1,4], R. D. BLAKELY[1,4];
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Abstract: Beginning with discovery of hyperserotonemia in a significant number of Autism Spectrum Disorder (ASD) patients more than 60 years ago, altered CNS and peripheral serotonin (5-HT) signaling has been proposed to contribute to ASD traits. A major determinant of extracellular 5-HT actions is the plasma membrane 5-HT transporter (SERT). We generated mice expressing the SERT coding substitution Gly56Ala (SERT Ala56 mice), originally found in subjects with ASD. In this model, we observed hyperserotonemia, increased rates of CNS 5-HT clearance, alterations in 5-HT signaling, deficits in social behavior and juvenile communication, as well as repetitive behavior and GI dysfunction. The constitutive hyperfunction of SERT Ala56 is mirrored acutely by the ability of IL-1β to enhance SERT activity through a p38α MAPK pathway *in vitr*o and is supported by p38α MAPK hyperphosphorylation *in vivo*. Moreover, both genetic elimination of p38a MAPK in 5-HT neurons and pharmacological treatment of MW150, a specific, brain penetrant p38α MAPK inhibitor, can normalize disrupted behavioral and GI traits. As 5-HT has been reported to diminish microglial reactivity, we hypothesize that excess 5-HT clearance of SERT Ala56 could lead to a tonic inflammatory state and contribute to ASD-like traits. We thus, performed qPCR analysis to quantify inflammatory cytokine levels in multiple brain regions of adult WT and SERT Ala56 mice. To assess inter-lab variability in these measures, we utilized tissues from colonies of SERT Ala56 mice housed at either FAU or Columbia University. Midbrain tissue from male SERT Ala56 mice from both colonies demonstrated a statistically significant elevation of IL-1β mRNA compared to WT, whereas neither IL-6 nor TNFa mRNA levels were different. Prefrontal cortex (PFC) samples derived from Columbia University demonstrated significant elevations in IL-1β and TNFα, but not IL-6, with nonsignificant elevation observed in FAU samples. No genotype effect was observed for hippocampal IL-1β, TNFα, or IL-6. In a small set of female midbrain samples, we detected elevated TNFa mRNA that was absent in males. Our findings indicate that male SERT Ala56 mice display evidence of region-specific, chronic inflammation, particularly as IL-1β elevations were reproducible across laboratories. The latter finding is noteworthy given the high level of IL1β receptors on serotonergic raphe neurons. Ongoing studies seek to evaluate further the sex-specificity of these findings, explore additional molecular and cellular markers of immune and glial activation, and determine whether basal inflammatory changes support altered responses to challenge with environmental stressors.

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Poster

604. Autism: Models

Location: SDCC Halls B-H

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Program #/Poster #: 604.12

Topic: A.07. Developmental Disorders

Title: Early corticostriatal hyperconnectivity in Shank3B-/- striatal SPNs is not caused by cell autonomous loss of Shank3.

Authors: *Y.-C. SHIH[1], R. PEIXOTO[2];
[1]Univ. of Pittsburgh Ctr. For Neurosci., Pittsburgh, PA; [2]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Early corticostriatal hyperconnectivity in *Shank3B*$^{-/-}$ striatal SPNs is not caused by cell autonomous loss of Shank3.
Accelerated maturation of corticostriatal synapses onto striatal spiny projection neurons (SPNs) is associated with the onset of behavior abnormalities in *Shank3B*$^{-/-}$ mice, a model of autism spectrum disorders (ASD). Previous studies have shown that global deletion of Shank3 results in cortical hyperactivity during early postnatal development. In addition, experimental manipulations that increase cortical activity during this period are sufficient to increase corticostriatal connectivity, suggesting that the phenotype observed in *Shank3B*$^{-/-}$ SPNs might be due to extrinsic changes in network activity. An alternative possibility is that loss of Shank3 causes cell-autonomous changes in intracellular signaling mechanisms that alter synaptic maturation. To test this hypothesis, we developed a conditional knockout protocol to specifically delete *Shank3* in a sparse number of SPNs in dorsomedial striatum, which is critical for learning and execution of motor actions and receives strong input from prefrontal cortex. This strategy allows us to examine the cell autonomous effect of Shank3B$^{-/-}$ during early development (P8-P15). By comparing wild-type and neighboring Shank3B$^{-/-}$ SPNs in the same individual animal, we found no difference of spontaneous miniature excitatory synaptic current between control cells and Shank3B$^{-/-}$ cells. Also, there was no sex difference. These results support the hypothesis that corticostriatal hyperconnectivity in early developmental Shank3B$^{-/-}$ mice is not caused by cell autonomous loss of Shank3, but is instead caused by increased network activity.

Disclosures: Y. Shih: None. **R. Peixoto:** None.

Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.13

Topic: A.07. Developmental Disorders

Title: Single-nucleus RNA-seq on mutant Wac mice provides initial insights into molecular and developmental mechanisms relevant to DeSanto-Shinawi Syndrome.

Authors: *N. SEBAN[1], C. P. CANALES[1], S. A. LOZANO[1], K. CICHEWICZ[1], A. M. STAFFORD[2], D. VOGT[2], J. ZHU[1], R. ORTIZ[1], M. COREA[1], D. RAHBARIAN[1], A. S. NORD[1]; [1]Univ. of California Davis, Davis, CA; [2]Pediatrics and Human Develop., Michigan State Univ., Grand Rapids, MI

Abstract: Rare monogenic syndromes are emerging as a collectively common cause of neurodevelopmental disorders associated with cognitive, social, behavioral, and neurological impacts, including autism, attention deficit hyperactivity disorder (ADHD) and seizures. One such example, DeSanto-Shinawi Syndrome (DESSH), is caused by mutations in the *WAC* gene. Previous reports have demonstrated a multitude of cellular roles for the WAC protein, including positive regulation of mammalian target of rapamycin (MTOR), mitosis, transcriptional initiation and autophagy. Using a constitutive murine *Wac* mutant mice that exhibits behavioral, anatomical, and cellular phenotypes relevant to humans diagnosed with DESSH, we evaluated transcriptional profiling at single-cell level for the forebrain at postnatal day (PND) 2. Our single-nucleus model consists of approximately 40,000 high quality cells that met our stringent QC standards, and over 20 clusters were assigned cell-type classifications based on canonical markers of cell identity. We found differential regulation across numerous cell populations. Including across broad neuronal classes such as glutamatergic or GABAergic clusters, as well as layer specific neuron populations and interneuron subtypes. Preliminary analysis also shows evidence for perturbed GABAergic signaling in line with interneuron histological changes seen in ongoing functional studies of the *Wac* mutant mice. Overall, our results provide novel insights regarding molecular and cellular pathologies associated with *Wac* haploinsufficiency. Future work is needed to understand how cell-type specific perturbations might drive pathology and to understand the molecular mechanisms of WAC protein that are required in the brain.

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Poster

604. Autism: Models

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Program #/Poster #: 604.14

Topic: A.07. Developmental Disorders

Support: NIH/NIAAA Grant R01027766

Title: Altered Adult Hippocampal Neurogenesis in the Absence of MEF2C Underlies Autism-like Phenotype in Mice

Authors: *S. BASU, Z. LIU, H. SUH;
Neurosci., Lerner Res. Institute, Cleveland Clin., Cleveland, OH

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder causing significant social, communication and behavioral deficits. Although genetic and environmental factors play a crucial role in ASD development, the precise cellular substrate associated with ASD has not been elucidated. Hippocampal adult neurogenesis (AN), a process where functional neurons are continuously generated and integrated in neural circuits in the post-natal brain, plays a critical role in structural and functional plasticity throughout life, and altered AN has been implicated in ASD. Till date, a handful of studies have addressed the role of regulatory molecules for AN in the ASD phenotype. Myocyte enhancer factor 2c or MEF2C is an activity-dependent transcriptional factor. A microdeletion of MEF2C is associated with ASD phenotype and altered MEF2C function has been linked with abnormal synaptic plasticity. Considering the functional association of MEF2C with ASD and the involvement of AN in ASD, we tested whether MEF2C has a critical role in ASD by regulating AN. In this study, we specifically deleted MEF2C in hippocampal newborn neurons, referred to as dentate granule cells (DGCs), using a cre-expressing retrovirus and investigated the role of MEF2C in the structural development of DGCs over time. In addition, we genetically deleted MEF2C in hippocampal newborn DGCs in double transgenic mice carrying ASCL1-CreER; $Mef2c^{fl/fl}$ alleles (MEF2C-cKO) and determined the MEF2C function in ASD-like behaviors, including sociability, cognition and anxiety. MEF2C deletion in hippocampal newborn DGCs reduced the dendritic length and arborization of the DGCs at 4, 8 and 12 weeks of neuronal ages. Dendritic spine densities of MEF2C-deficient DGCs did not change at 4 weeks, but were increased in 8 and 12 weeks old DGCs, respectively, suggesting a crucial role of MEF2C in synapse elimination. In line with these structural deficits, MEF2C-cKO mice failed to discriminate between novel and familiar mice in the 3-chamber sociability test, as well as showed impaired contextual fear memory. However, MEF2C-cKO mice did not show significant differences in object recognition memory and anxiety compared to control mice. Taken together, our study showed the essential role of MEF2C in the development of hippocampal newborn neurons and impaired structural development and synaptic connectivity of newborn DGCs may underlie ASD. Thus raising the possibility that AN may serve as a therapeutic target to diagnose, treat, and prevent ASD.

Disclosures: **S. Basu:** None. **Z. Liu:** None. **H. Suh:** None.

Poster

604. Autism: Models

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Topic: A.07. Developmental Disorders

Support: National Institute of Health (NINDS) NS82635
SLC6A1 Connect, Taysha Gene Therapies
NIH R01NS 121718
NIH Rise Grant

Title: Haploinsufficiency resulting from a proximal microdeletion of SLC6A1 and SLC6A11 associated with 3p- syndrome, epilepsy, and neurodevelopmental delay, and proposed 4-phenylbutyrate rescue

Authors: *M. DELEEUW[1,2], W. SHEN[4], J.-Q. KANG[3];
[1]Meharry Med. Col. Neurosci. and Pharmacol., Nashville, TN; [2]Dept. of Neurol., [3]Dept Neurol., Vanderbilt Univ., Nashville, TN; [4]Dept. of Neurol., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: GABA transporters are responsible for the reuptake of γ-aminobutyric acid (GABA) from the synaptic cleft. GABA transporter 1 (GAT-1), encoded by *SLC6A1*, is expressed in GABAergic neurons and astrocytes, and GABA transporter 3 (GAT-3), encoded by *SLC6A11*, is expressed in astrocytes. Mutations in *SLC6A1* are associated with a wide spectrum of neurodevelopmental disorders, such as epilepsy syndromes, intellectual disabilities, and autism. While the association of *SLC6A11* with disease is uncertain, both genes are located at the same 3p25.3 region. A proximal microdeletion of *SLC6A1* and *SLC6A11* has been reported and is recurring; however, the functional consequence of the loss of GAT-1 and GAT-3 is unknown, nor the differential contribution to GABA uptake. The goal of this research is to characterize the functional consequence of the microdeletion of *SLC6A1* and *SLC6A11* related to 3p- syndrome and a possible rescue with a pharmacochaperoning approach such as 4-phenylbutyrate acid (PBA). ^{3}H radiolabeling was used to measure GABA uptake and respective transporter expression to evaluate the impact of the microdeletion using mouse cortical astrocytes and HEK293T cell lines that express recombinant GAT-1 and GAT-3 transporters. To mimic haploinsufficiency, the experimental effect of a half gene dose was determined through the transfection of a mixture of GAT-1 or GAT-3 cDNAs with pcDNA. Transfected cells were treated with Cl-966 (50μM), SNAP5114 (30μM) to determine the specific GAT uptake activity. Cells were treated with PBA to determine the chaperone's effect on GABA uptake as a potential treatment option. Preliminary data confirmed that the loss of a half gene dose due to the microdeletion of *SLC6A1* and *SLC6A11* resulted in reduced GABA uptake. With specific GAT-1 or GAT-3 inhibitors, we identified that GABA uptake from both transporters is reduced. Pharmacochaperoning approach with PBA (2mM, 24 hrs) increases the GABA uptake in both wildtype and microdeletion condition. Preliminary patient data shows improvement in seizure activity and neurodevelopmental delay following PBA treatment. The observed reduction in GABA reuptake resulting from haploinsufficient copies of *SLC6A1* and *SLC6A11* would account for the observed phenotype, as all reported cases to-date exhibit seizure activity and neurodevelopmental delay. Given that these symptoms are prominent features of patients with point mutations in *SLC6A1* alone, this study suggests that the proximal microdeletion of both *SLC6A1* and *SLC6A11* is likely to contribute to a great extent, perhaps causative for the observed phenotype.

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Poster

604. Autism: Models

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Program #/Poster #: 604.16

Topic: A.07. Developmental Disorders

Support: NSF NRT Grant DGE-1633516

Title: Automated fluorescence image analysis to investigate the altered development of the ganglionic eminence along spatial and cell type dimensions in the engrailed 2-knockout mouse

Authors: *J. S. MARTINEZ-FUENTES[1], M. COGSWELL[2], A. C. CASTELLÓ[3], V. BORRELL[4], S. J. RUSSEK[5];
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Abstract: A variety of genetic and environmental factors contribute to autism spectrum disorder (ASD), but exactly how this occurs remains unclear. One hypothesis is that factors may converge to perturb the development and function of the gamma-aminobutyric acid (GABA) system of the brain. The midbrain-hindbrain patterning homeotic transcription factor engrailed 2 (En2) has been associated with ASD in humans, and a mouse model harboring a null mutation of En2 recapitulates behavioral and neuroanatomical abnormalities reminiscent of ASD as well as epilepsy. Interestingly, En2-null mice show reduced GABAergic marker expression in the hippocampus and cortex. How En2 dysfunction leads to neuropathological phenotypes remains unknown. Our recent *in vivo* work draws attention to the potential role of En2 in radial glial self-renewal and subsequent differentiation, implicating the birth of GABAergic interneurons and spiny projection neurons. To aid investigation into the role of En2 in the development of forebrain GABAergic neurons, we are assessing the cell and molecular alterations that may occur at the ganglionic eminence (GE, age E13), the major birthplace of inhibitory neurons, in En2-null mice compared to wild-type (WT) controls. We hypothesize that, in line with our *in vivo* work, loss of *EN2* function leads to a decrease in the apical radial glia population but a relative increase in the proportion of postmitotic neurons, at least at the lateral GE region. Here, we use sensitive multiplexed fluorescence *in situ* hybridization with high-resolution fluorescence microscopy to track spatial distributions of WT *En2* transcript, and to track transcriptionally defined subtypes of progenitor cells based on overlapping expression of *Gsx2* and *Ascl1* at GE regions (i.e., medial, lateral, caudal GE) across WT and En2-null conditions. To this end, we apply MATLAB automated image analysis to quantify transcript signal in thousands of individual cells along spatial and cell type dimensions. Data show that *En2* is regionally widespread cross the LGE (~70% cells express at least one *En2* transcript), but sparse in its density (~96% of *En2*+ cells express <5 transcripts). Interestingly, ongoing results at the LGE suggest enrichment of high-expressing *En2*+ cells in the ventral subregion, potentially indicating vulnerability of cells from this area to progenitor dysfunction in the En2-null condition. Taken together, widespread but sparse presence of *En2* suggests this factor may play a role in cells arising from the GE, raising questions as to how En2 dysfunction along GABAergic maturation trajectories may contribute to etiology of ASD and other developmental disorders of brain function.

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Poster

604. Autism: Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.17

Topic: A.07. Developmental Disorders

Support: NIHR21MH126400
NIGMSR35GM119831

Title: Functional screening of autism associated cis regulatory mutations

Authors: J. T. LAMBERT[1], ***A. SANTOS**[1], K. CICHEWICZ[1], L. SU-FEHER[1], T. L. WARREN[1], J. HAIGH[2], S. MORSE[1], J. HANNERS[1], E. CASTILLO PALACIOS[1], A. NORD[1]; [1]Univ. of California - Davis, Davis, CA; [2]Univ. of Leeds, Leeds, United Kingdom

Abstract: The precise timing of gene expression is crucial for proper development. The non-coding genome, which accounts for as much as 80% of the human genome, plays regulatory roles such as maintaining cell-type and spatiotemporal specific gene expression. Not surprisingly, mutations in the regulatory genome have been associated with neurodevelopmental and neurological disorders. However, deciphering which of these mutations are relevant for disease manifestation remains challenging. Here we address this using a massively parallel reporter assay (MPRA) to identify cis-regulatory elements (CREs) active during early post-natal development that may be relevant to autism spectrum disorders (ASD). We selected ~1000 candidate CREs centered around de novo mutations identified in ASD probands and siblings. Mutations were selected from the Simons Simplex Collection based on predicted brain enhancer activity from epigenomic datasets, prioritizing mutations predicted to disrupt transcription factor binding motifs. Our library included reference and mutant sequences representing 855 mutations from probands and 339 mutations from unaffected siblings. Using a modified STARR-seq design, our library was then packaged into AAV and delivered by intracranial injection into the mouse brain at post-natal day 0 (P0). Dorsal forebrain was collected at P7 when DNA and RNA were extracted for sequencing. Sequencing quality was good and correlation of reads per amplicon across replicates high. Input-normalized activity estimates for each candidate enhancer were generated by taking the RNA/DNA ratio and we observed high reproducibility in activity measures across replicates. Of the CREs tested, we identified 173 sequences that consistently increased reporter expression. We found a 1.31-fold enrichment in the proportion of proband mutation sequences that were MPRA-defined enhancers. We also identified altered activity levels between variant and reference alleles within our MPRA library for experimental validation. Interestingly, we detected a higher overall positive rate among ASD probands compared to unaffected sibling candidate CREs, further suggesting our results have relevance to ASD.

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Poster

604. Autism: Models

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 604.18

Topic: A.07. Developmental Disorders

Title: Neurodevelopmental phenotype of CLC-4 knock out mice: A model for Autism Spectrum Disorder

Authors: *S. LEE[1], S. JEON[2], J. HAN[3], Y. KIM[1];
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Abstract: Mutation in chloride voltage gated channel-4 (CLC-4), a subtype highly expressed in the brain, is associated with X-linked intellectual disability, seizure disorders and autism spectrum disorder (ASD) in humans. It was previously postulated that as CLC-4 KO mice had no neurodevelopmental phenotype because CLC-3 played the main role in the brain. We investigated the neurodevelopmental development, three chamber paradigm test and marble burying test in CLC-4 Knockout (KO) mice. CLC-4 KO mice showed impairment in social interaction and increase in stereotypic behavior, consistent with clinical features in ASD. The expression level of PSD95, the phosphorylation levels of synapsin in Ser9, Ser549, and Ser609 and the activation levels of CREB and ERK activation were significantly reduced in the cortex and hippocampus of CLC-4 KO mice compared to wildtype (WT). In the Sholl analysis, the number of dendrite branching was significantly decreased in CLC-4 KO cortical neurons compared to WT. Risperidone decreased the stereotypic behavior in marble burying tests and improved the social impairment in three chamber tests. Risperidone also recovered the reduced expression of CDK5 and PSD95, and the phosphorylation of synapsin on Ser9 and Ser549 in the cortex of CLC-4 KO mice. Risperidone restored the number of MAP2 positive cells and the number of dendrite branching in CLC-4 KO neurons. In conclusion, CLC-4 KO mice show neurodevelopmental endophenotypes consistent with human developmental disabilities and Risperidone, a drug commonly used in ASD for behavioral control, improved the endophenotypes while recovering the molecular changes associated with the phenotype.

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Poster

604. Autism: Models

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Program #/Poster #: 604.19

Topic: A.07. Developmental Disorders

Support: K01NS107723

Title: Developmental expression and function of integrin β3 in mouse cerebral cortex and hippocampus

Authors: H. N. COOK, H. V. RODRIGUEZ, C. J. HANDWERK, A. R. KALINOWSKI, C. J. DENZLER, *G. S. VIDAL;
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Abstract: A strong positive association exists between mutations in integrin β3 (*Itgb3*) and autism spectrum disorder. Integrins (a class of heterodimeric cell adhesion molecules) are required for normal structural development of dendrites and synapses. The function of integrin β3 in cerebral cortical and hippocampal neurons and glia in vivo was unknown until recent studies from our lab showing that *Itgb3* is required for normal dendritic arborization of layer II/III pyramidal neurons in vivo in a cell-specific manner (https://doi.org/10.1186/s13041-020-00707-0), and that it is required in forebrain excitatory neurons and astrocytes for normal social and grooming behaviors in mice (https://doi.org/10.1186/s12868-022-00691-2). These and other prior studies suggest that integrin β3 is required in neurons of the cortex and hippocampus for normal brain function, and that its postnatal expression could be developmentally regulated. However, *Itgb3* expression in cerebral cortex and hippocampus is not well understood. We set out to characterize *Itgb3* expression utilizing fluorescent in situ hybridization (RNAscope), which localized *Itgb3* transcripts throughout the cortex and hippocampus of early postnatal and juvenile mice. Double fluorescent immunohistochemistry was used in conjunction with RNAscope to assess cell type-specific expression of *Itgb3*. Preliminary results suggest a strong laminar pattern of *Itgb3* expression in layer V of the cortex, and a pattern of expression strongly restricted to stratum pyramidale of hippocampal region CA3. We designed loss of function studies to assess a potential role for Itgb3 in the dendritic development of pyramidal neurons in cortical layer V and hippocampal region CA3. Results from these studies show differential roles in regulating the development of dendritic structure in cortex and hippocampus. Taken together, results suggest that integrin β3 could serve multiple roles in developing cortical and hippocampal excitatory circuitry.

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Poster

604. Autism: Models

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Program #/Poster #: 604.20

Topic: A.07. Developmental Disorders

Support: NRF-2021R1C1C1008231

Title: A novel heterozygous UBA6 point mutation in a patient with autism spectrum disorders and epilepsy

Authors: J. LEE[1], ***M.-J. KIM**[2], I.-H. JEONG[3], M.-S. YUM[2], P. C. LEE[3];
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Abstract: Advanced next-generation sequencing and human genetics helped to identify a number of new pathogenic genetic causes that are associated to autism spectrum disorder (ASD) and the ubiquitin proteasome system (UPS) is highlighted one of these genetic causes of ASD. We previously identified that the neuronal loss of UBA6, which constitutes an independent arm of the UPS led to decreased dendritic spine density and behavior dysfunction in mouse model. Here, we report the first clinical case that can support the association of UBA6 in human brain development. To identify the genetic mutation in female patients with ASD, microcephaly, and seizure, whole exome sequencing and chromosomal microarray from whole blood sample. Discharge and in vitro ubiquitylation assays were performed for functional validation of de novo *UBA6* mutation. Using whole exome sequencing data analysis, heterozygous novel de novo mutation of *UBA6* (c.G1921T, p.A641S) were detected in female patient who had been represented characteristic clinical features of ASD, microcephaly and seizure. In vitro functional analysis, this de novo *UBA6* (c.G1921T, p.A641S) mutation results in decreasing level of UBA6 protein and loss of catalytic activity of UBA6 protein. Additionally, the ubiquitin transferring system to UBE3A via UBA6-USE1 pathway also disrupted. This is the first human case report of the patients with de novo *UBA6* mutation presenting severe ASD and epilepsy which suggests the critical role of UBA6 in brain development. Although this one case is not complete to prove the pathogenicity of *UBA6*, these results would begin to outline the functional role of UBA6 in human brain development.

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Poster

604. Autism: Models

Location: SDCC Halls B-H

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Program #/Poster #: 604.21

Topic: A.07. Developmental Disorders

Support: Department of Human Genetics

Title: Ash1l Loss-of-Function Alters Cortical Development in Mouse

Authors: *K. P. TOOLAN, M. L. BRINKMEIER, S. L. BIELAS, S. A. CAMPER;
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Abstract: Over 100 genes have been implicated in the genetic etiology of autism spectrum disorder (ASD). Many high confidence ASD genes are involved in chromatin remodeling, histone modifications and DNA methylation. Among these is *ASH1L* (Absent, Small, Homeotic-Like 1), which catalyzes histone H3K36 methylation and has a role in activating transcription by counteracting polycomb repression. 97% of individuals with heterozygous *ASH1L* loss of function variants have ASD, intellectual disability, and/or speech delay. 66% of the patients have craniofacial or eye abnormalities, and skeletal, heart, and/or genital anomalies are reported. *However, the link between ASH1L variants and the associated spectrum of neurodevelopmental and structural defects is poorly understood.* We utilized germline (*Ash1l*$^{-/-}$, p.V1693Afs*2) and cortical conditional (*Emx1-Cre*^{ERT2}*;Ash1l*$^{fl/fl}$) knockout mouse models to address this. *Ash1l*$^{-/-}$ mice are not viable after birth, and at E18.5 there is no evidence of macro- or microcephaly (total N=42, p=0.79), but the nasal bone is shorter (N=5-6/genotype, p=0.01). The mutant brains did not have any obvious dysmorphology, and L1cam immunostaining indicated that the corpus callosum was normal (N=3/genotype). We induced deletion of *Ash1l* in cortical excitatory neurons by injecting pregnant dams with tamoxifen at E10.5 and analyzed brain morphology at E18.5. There were no obvious differences in brain size or morphology between *Emx1-Cre*^{ERT2}*;Ash1l*$^{fl/fl}$ and control littermates (N=24-27/genotype, p=0.08). We performed single-cell RNA sequencing of cortical tissue from E18.5 *Emx1-Cre*^{ERT2}*;Ash1l*$^{fl/fl}$ (N=4) and control littermates (N=3) that received tamoxifen at E10.5. After quality control, excitatory neuronal cells were clustered based on gene expression, and clusters were identified based on accepted cell-type specific markers. Most cortical neuron subtypes are present in mutants in numbers comparable to controls. However, migratory neurons and a subset of upper layer neurons make up 16.9% and 15.9% of cells, in the controls, respectively, but only 0.5% and 0.3% in the mutants. An ASH1L-dependent transitional cell type is present, comprising of 47.5% of the total cell population in mutant cortices. Taken together, these gene expression data indicate that ASH1L has an important role in maturation of upper layer, cortical neurons. Our *Ash1l* mouse models reveal the broad impact of ASH1L on the development of craniofacial structures including the brain, providing the opportunity to uncover pathogenic mechanisms that underlie these structural and functional defects.

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Poster

604. Autism: Models

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Topic: A.07. Developmental Disorders

Support: Autism Center of Excellence Grant awarded by the National Institute of Child Health and Development (NICHD) (P50 HD093079)

Title: Shedding light on the genetic drivers of disproportionate megalencephaly in autism spectrum disorder

Authors: *A. SHCHERBINA[1], S. T. SCHAFER[2], C. NARAYANAN[1], S. BANERJEE[1], S. CHETTY[1,3];
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Abstract: Several reports in the literature associate disease severity of Autism Spectrum Disorder (ASD) with increased rate of head growth in early childhood. Though the presence of disproportionate megalencephaly is likely associated with abnormal processes during development, the genetic basis of the association with ASD remains poorly understood. An increase in brain size often precedes the first clinical signs of the disorder, suggesting that understanding the mechanisms leading to brain overgrowth could provide a window of opportunity to intervene and possibly prevent disease onset. Here, we develop an in vitro model using human induced pluripotent stem cells (hiPSCs) to investigate the mechanisms regulating cortical neural progenitor cell (NPC) development in ASD. In particular, we derive iPSCs from control and patient groups with typical development and normal brain size (TD-N), with ASD diagnosis and normal brain size (ASD-N), and with ASD diagnosis with disproportionate megalencephaly (ASD-DM).
Transcriptomic analysis of four biological replicates within each group identifies signatures that differentiate the ASD-N from TD-N as well as signatures that differentiate ASD-DM from ASD-N. Biological processes associated with a metabolic shift from oxidative phosphorylation to glycolysis (glucose-6-phosphate mediated glycolytic processes, NADH regeneration, glucose catabolic process to pyruvate) were found to be significantly upregulated within the ASD-DM cohort relative to ASD-N, driven by upregulation of the PKM, PGK1, GAPDH, ALDOA, and PGAM1 genes. Comparisons of the ASD-DM phenotype to TD-N revealed upregulation of immune-related signaling pathways driven by the CD99 pathway, Interferon-beta, type-I interferon, interleukin-5, and interleukin-7. The ASD-DM vs TD-N comparison also highlights upregulation of cell cycle and proliferation as well as DNA damage repair pathways that have previously been associated with cancer (driven by N4BP2 and HNRNPK). Meanwhile, the ASD-N signature was characterized by enrichment of biological processes associated with negative regulation of neuron differentiation, positive regulation of apoptosis, and negative regulation of the immune response.
These results highlight the genetic drivers of differences between ASD-DM and ASD-N phenotypes, suggesting implication of metabolic processes associated with the Warburg effect, interferon and interleukin signaling pathways, and regulation of cell proliferation pathways that have previously been associated with cancer.

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Poster

604. Autism: Models

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Topic: A.07. Developmental Disorders

Support: SFARI Pilot Award
NIH 5R01NS104055-02

Title: Asd gene Bcl11a/Ctip1regulates subcellular rna localization by projection neurons, developing associative circuitry, and social behavior

Authors: *O. DURAK[1], J.-Y. KIM[1], D. E. TILLMAN[1], Y. ITOH[1], M. WETTSTEIN[1], L. C. GREIG[1], J. D. MACKLIS[2];
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Abstract: Precise, area-specific connectivity of interhemispheric callosal projection neurons (CPN) in the cerebral cortex is critical for sensory, associative, and behavioral functions. CPN circuitry, which connects and integrates the two cerebral hemispheres via the corpus callosum, is centrally implicated in autism spectrum disorder (ASD) and intellectual disability (ID). Though transcriptional controls regulating CPN subtype and areal development have progressively become partially understood, downstream subcellular mechanisms and molecular machinery that implement precise and diverse CPN circuitry is essentially unknown.Here, we identify that the highly penetrant ASD/ID risk gene *Bcl11a/Ctip1* is critical developmentally for appropriate and precise areal targeting of CPN associative projections, and that its deletion both strikingly reshapes these projections, and causes dramatic disruption to circuit-specific subcellular growth cone (GC) molecular machinery, social behavior, and cognition in mice. CPN-specific embryonic deletion of *Bcl11a* causes loss of correct homotopic targeting in the contralateral cortex, re-routes a substantial proportion of their axonal projections through the evolutionarily older anterior commissure, and induces strikingly aberrant, but specific and precise, projections to the basolateral amygdala in adult mice. Importantly, bilateral deletion of *Bcl11a* from CPN alone results in abnormal social behavior and working memory. Mechanistically, we identify dysregulation of the CPN axonal GC-localized transcriptome in *Bcl11a* nulls, due to either aberrant transcription or trafficking of individual transcripts. These molecular mis-localizations disrupt axon guidance and CPN-specific associative circuitry formation, causing disease-related behavior. Together, this work identifies critical functions for *Bcl11a* in CPN axonal connectivity, development of functional associative-social circuitry, regulation of subtype-specific subcellular molecular machinery *in vivo*, revealing novel GC-localized transcripts that regulate precise axonal targeting and circuit formation. These results elucidate development and ASD/ID disease-related circuit disruption of CPN, and the importance of understanding circuit-specific subcellular- e.g. GC vs. soma- localization of RNA and protein molecular machinery by neurons.

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Poster

604. Autism: Models

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Program #/Poster #: 604.24

Topic: A.07. Developmental Disorders

Support: NIH F30 Award MH127800

Title: Evidence from novel mouse model suggests that phenotype of ADNP syndrome may be influenced by site of mutation

Authors: *M. CONROW-GRAHAM, Z. YAN;
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Abstract: Activity-dependent neuroprotective protein (ADNP) is a top-ranking risk factor for autism spectrum disorder (ASD) and intellectual disability (ID). The hallmark of ADNP syndrome is prominent ASD/ID. Mice with heterozygous loss of function of Adnp exhibit growth impairment, developmental delays, cognitive problems, and synaptic dysfunction. However, there is evidence that the site of the mutation in *ADNP* may affect behavioral and functional phenotypes. Heterozygous mutations in human ADNP syndrome have been found throughout the length of the gene coding sequence, but most preclinical investigation has focused on the effect of Adnp loss-of-function. Here, we have investigated a novel mouse model, C57BL/6-*Adnp*^{em1Ant}/J (*Adnp*^{em1Ant}), with a mutation near the C-terminal domain of *Adnp*. *Adnp*^{em1Ant} carries a heterozygous 14 nucleotide deletion in exon 5, at amino acid 822, followed by a premature stop codon. This is predicted to lead to expression of a truncated protein, rather than undergoing nonsense-mediated decay. We find that, at 6 weeks postnatal, *Adnp*^{em1Ant} animals recapitulate some of the phenotypes seen in children with ADNP syndrome, including reduced body size and impaired visual function. Behaviorally, *Adnp*^{em1Ant} differs from mice with heterozygous loss of *Adnp*, in which the most prominent neurodevelopmental phenotype is ID-like cognitive dysfunction. Instead, the C-terminus mutation appears to induce predominant autism-like traits, including impaired sociability and increased grooming. Further, these behaviors show sexual divergence, with greater social impairment and repetitive grooming present in males. Gene expression and glial activation also differ from mice with postnatal Adnp deficiency in prefrontal cortex. These preliminary findings suggest that the consequences of site-specific mutations in *ADNP* merit further investigation, rather than assuming that all mutations lead to a deficiency or loss-of-function phenotype.

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Poster

604. Autism: Models

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Program #/Poster #: 604.25

Topic: A.07. Developmental Disorders

Support: National Honor Scientist Program (NRF-2012R1A3A1050385)

Title: Dysfunction of NMDA receptors in neuronal models of an autism spectrum disorder patient with a DSCAM mutation and in Dscam-knockout mice.

Authors: *M. KIM[1], C.-S. LIM[3], J. CHOI[1], M. ISLAM[1], Y.-K. LEE[4], K.-W. SHIM[2], J.-H. LEE[5], Y.-S. LEE[6], K. LEE[7], J.-A. LEE[4], B.-K. KAANG[1];
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Abstract: One of the neurodevelopmental disorder, autism spectrum disorders (ASD), shows heterogeneous pathologies so that ASDs require individualistic and patient-specific research. Recent improvement, human-induced pluripotent stem cell (iPSC) technology, provides a distinct platform for modeling ASDs to study complex neuronal phenotypes. Here, we generated telencephalic induced neurons (iNs) from iPSCs derived from an ASD patient with a heterozygous point mutation in the DSCAM gene. The mRNA of DSCAM and the density of DSCAM in neurites were significantly decreased in ASD compared to control iNs. RNA sequencing analysis showed that several synapse-related genes including NMDA receptor subunits were downregulated in ASD iNs. Moreover, NMDA receptor (R)-mediated currents were also reduced in ASD compared to control iNs. Normal NMDA-R-mediated currents were rescued by expressing wild-type DSCAM in ASD iNs, and reduced currents were observed by truncated DSCAM expression in control iNs. shRNA-mediated DSCAM knockdown in control iNs resulted in the downregulation of an NMDA-R subunit, which was rescued by the overexpression of shRNA-resistant DSCAM. In fact, DSCAM was co-localized with NMDA-R components in the dendritic spines of iNs whereas their co-localizations were significantly reduced in ASD iNs. A neural stem cell-specific Dscam heterozygous knockout mice show deficits in social interaction and social memory with reduced NMDA-R currents. These data suggest that DSCAM mutation causes pathological symptoms of ASD by dysregulating NMDA-R function.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant P01AG14449
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Title: Postnatal chemophenotypic neuronal alterations in the frontal cortex, hippocampus and cerebellum in Down syndrome

Authors: *S. E. PEREZ[1], D. G. MORENO[1], E. C. UTAGAWA[1], J. C. MIGUEL[1], N. C. ARVA[2], K. T. SCHAFERNAK[3], M. H. MALEK-AHMADI[4], E. J. MUFSON[1];
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Abstract: Down syndrome (DS) people display a reduction in frontal lobe (FC), hippocampal and cerebellar volume attributed to prenatal proliferation/migration deficits, however, the effect of trisomy 21 upon the postnatal neuronal development remains under-investigated. We examined the neuronal phenotypes within the FC, hippocampus and cerebellum obtained at autopsy from DS and neurotypically developing (NTD) neonates born at 28-weeks'-gestation up to 3 years of age using antibodies against non-phosphorylated neurofilament (SMI-32); calbindin D-$_{28k}$ (Calb), calretinin (Calr) and parvalbumin (Parv); neurogenesis doublecortin (DCX); proliferation Ki-67; amyloid precursor protein (APP)/beta-amyloid (Aβ); A$\beta_{1\text{-}42}$ and phosphorylated tau (p-tau, CP13 and PHF-1). Our findings showed a greater reduction in DS DCX-immunoreactive (-ir) cells in the FC and hippocampus. Both groups showed a similar distribution/number of SMI-32-ir cells in the hippocampus and cerebellum. FC SMI-32-ir cells appeared as early as 28'-weeks-gestation in NTD, but not until 196 weeks in DS. Although the distribution of Calb-ir neurons in the FC, hippocampus and cerebellum were similar between the youngest and oldest NTD and DS cases, the number of Calb-ir cells were significantly higher only in the NTD FC. Hippocampal Calr-ir cells and fibers were observed at all ages in DS, whereas Calr-ir fibers were mainly displayed in NTD cases. FC and cerebellar Calr-ir cell counts were comparable between groups. Parv-ir cells were found only in the cerebellum in DS and NTD. APP/Aβ-ir diffuse-like deposits were seen in the FC, hippocampus, and cerebellar cortex in DS and NTD. Only intraneuronal A$\beta_{1\text{-}42}$ immunoreactivity was detected in Purkinje cells in both groups. p-tau profiles were found in the FC and cerebellar cortex in the youngest DS and NTD cases. These findings suggest that trisomy 21 affects the neuronal chemophenotype in postnatal FC, hippocampus and cerebellum that may contribute to cognitive impairment in DS.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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UW Alzheimer Disease Research Center

Title: A Trisomy 21 Human Stem Cell Model of Basal Forebrain Cholinergic Neurons

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Abstract: Down syndrome (DS), or trisomy 21, is characterized by intellectual impairment at birth and Alzheimer's disease (AD) pathology in middle age. Basal forebrain cholinergic neurons (BFCNs) are a neuronal population critical for memory and cognition and are vulnerable to degeneration in both DS and Alzheimer's disease. The key molecular pathways involved in BFCN development and degeneration are poorly understood, but *in vitro* generation of BFCNs from human pluripotent stem cells (hPSCs) is a valuable method by which to investigate and identify these factors. Few differentiation protocols have been established to derive BFCNs from hPSCs. To address this methodological gap, we used a human embryonic stem cell line, H9, to develop a protocol that mimics key transcription factor expression in neural progenitor cells (NPCs) as they differentiate into BFCNs. BFCNs develop from LHX8 and ISLET1-expressing progenitors in the ventral telencephalon, and ultimately express choline acetyl transferase (ChAT) upon maturation. We first modified existing protocols with addition of SHH or SAG and validated that early addition of SHH results in robust NKX2.1 expression, indicative of ventralization. BFCN progenitor fate specification (LHX8 expression) was manipulated by addition of NGF at different timepoints. Results showed a time dependent increase in expression of LHX8, but no significant difference in the percentage of cells expressing LHX8. We also found that addition of BMP9 results in an increase in ISLET1 expression, indicating a progression toward mature BFCNs. Together these results provide a new strategy to differentiate hPSCs to BFCNs. To determine whether there are differences in the generation of BFCNs in DS, we built ChAT-P2A-Cre mCherry reporter lines in an isogenic pair of Ts21 and control iPSCs. We then employed our newly developed protocol to generate BFCN neurons (NeuN+/ChAT+) in both lines, and we found that Ts21 iPSCs generated less NeuN+ neurons as compared to isogenic controls. These results suggest that BFCNs in DS may have altered development that results in fewer BFCNs, which in turn may affect the impact of their neurodegeneration as fewer BFCNs will be spared. Here we provide a new model to explore the differences of BFCNs derived from

control and disease specific hPSCs. This platform will enable mechanistic studies on BFCN susceptibility in disease.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.03

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Title: DYRK1A inhibitor Leucettinib-21 targets cellular deficits in human trisomy 21 iPSC-derived neural cells

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Abstract: Down syndrome (DS, trisomy 21), caused by a full or partial trisomy of chromosome 21 (Hsa21), is the leading genetic cause of intellectual disability. Dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A), an Hsa21 encoded gene, is overexpressed in cells of individuals with DS and plays a role in cell cycle regulation, neurogenesis, outgrowth of neurites, and neuronal trafficking. Leucettinib-21 is a potent and selective, low-molecular weight, pharmacological inhibitor of DYRK1A, currently undergoing regulatory preclinical studies. It is developed as a drug candidate to correct cognitive disorders associated with DS. Leucettinib-21 has shown promise for improving cognition in animal models of DS. We tested the effects of Leucettinib-21 (LCTB-21) or iso-Leucettinib-21 (iso-LCTB-21), its kinase inactive isomer, on DYRK1A activity in human trisomy 21 neural cells. Neural progenitor cells (NPCs) and neurons were generated from an isogenic pair of trisomy 21 (Ts21) and control induced pluripotent stem cells. We confirmed that Ts21 NPCs and neurons have increased expression of DYRK1A protein compared to controls. LCTB-21 reduces the activity of DYRK1A in a dose-dependent manner, however, DYRK1A expression is stable across time in NPCs and neurons. LCTB-21 initially inhibits phosphorylation of Cyclin D1 at T286, a DYRK1A-specific site, in

Ts21 and control NPCs with levels of pT286-CycD1 steadily returning to baseline. Total Cyclin D1 is increased in Ts21 and control NPCs and neurons, a consequence of its dephosphorylation-induced stability. LCTB-21 preferentially targets DYRK1A and has no effect on the level or activity of GSK3. No significant changes in proliferation of Ts21 NPCs were observed within the 24-hour time frame that was assessed. Ts21 neurons have fewer synapsin-positive synapses than controls, and LCTB-21 led to an increase of synapses in Ts21 neurons while there was no effect on controls. In summary, LCTB-21 targets and inactivates DYRK1A in a dose-dependent manner in human Ts21 iPSC-derived NPCs and neurons while the kinase inactive isomer has no effect. LCTB-21 does not increase NPC proliferation but does partially rescue the decreased synapses in Ts21 neurons, providing valuable data for the therapeutic potential of LCTB-21.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.04

Topic: A.07. Developmental Disorders

Support: NIH Grant AG056850
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Title: Altered microglia differentiation in Down syndrome

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Abstract: Our lab and collaborators have reported increased inflammatory molecule levels and altered microglia morphology in brains of young adults and even children with Down syndrome (DS), a genetic disorder caused by the presence of a partial or fully triplicated copy of chromosome 21. However, it is still unknown when microglia alterations emerge in DS brains. Here, we explore microglia morphology and function in human prenatal cortical tissue and in the Dp(16)1Yey/+ (Dp16) mouse model of DS. To characterize microglial morphology, we performed immunofluorescence (IF) and image analysis using the microglia-specific marker

IBA1. In human euploid prenatal cortical tissue, we observed exclusively amoeboid microglia, which characterizes the earliest stages of microglia differentiation in the developing mammalian brain. In contrast, in trisomy 21 prenatal cortical tissue, we observed both amoeboid and intermediate stage microglia, exhibiting typical short processes. To evaluate microglial function, microglia cultures from euploid and trisomic prenatal cortical tissue suspensions were established. Phagocytic function was tested using fluorescent bioparticles and IBA1 immunostaining. Trisomic microglia displayed significantly increased particle engulfment per cell compared to euploid cultures. In the Dp16 mouse model, we observed that microglia from both cortex and hippocampus exhibited significant increases in surface area and branch length compared to wildtype controls at postnatal day (P)9. One possibility is that these early phenotypic alterations represent an advanced developmental stage, raising the possibility of an accelerated microglial differentiation phenotype associated with chronic inflammation in DS brains. We are currently analyzing cell-specific gene expression profiles to further characterize microglial structural and functional differences in the trisomic brain. Thus far, the results suggest that early interventions during development may be critical to preventing chronic inflammation and microglial abnormalities in DS.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: R01HD090180

Title: Genetic modeling and analysis of Down syndrome in mice

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Abstract: Down syndrome (DS) is an important genetic cause of cognitive deficits and hearing impairment. The mouse remains an essential model organism in DS research because human chromosome 21 (Hsa21) is orthologously conserved with three regions in the mouse genome. To model DS, we have engineered many mouse models of DS which carry the triplications of different Hsa21 orthologous regions. By using a coat color transgene, we have developed the strategy to identify the desired mouse mutants efficiently. The mutant mice selected via coat colors and DNA-based genotyping were used for the studies on the phenotypes, including hearing impairment. The clinical manifestations of hearing impairment have been attributed to

multiple factors. Mouse models could provide mechanistic insights on various causes of hearing loss in DS. To investigate mechanisms of hearing loss in DS in the absence of the cadherin 23 mutation, we backcrossed our DS mice, Dp(16)1Yey, onto normal-hearing CBA/J mice and evaluated their auditory function. Distortion product otoacoustic emissions (DPOAE), a test of sensory outer hair cell (OHC) function negatively impacted by conductive hearing loss, were reduced in amplitude and sensitivity across all frequencies in DS mice. The middle ear space in DS mice appeared normal with no evidence of infection. MicroCT structural imaging of DS temporal bones revealed a smaller tympanic membrane diameter, oval window, and middle ear space and localized thickening of the bony otic capsule, but no gross abnormalities of the middle ear ossicles. Histological analysis of the cochlear and vestibular sensory epithelium revealed a normal density of cochlear and vestibular hair cells; however, the cochlear basal membrane was approximately 0.6 mm shorter in DS than WT mice so that the total number of hair cells was greater in WT than DS mice. In DS mice, the early and late peaks in the auditory brainstem response (ABR), reflecting neural responses from the cochlear auditory nerve followed by subsequent neural centers in the brainstem, were reduced in amplitude and ABR thresholds were elevated to a similar degree across all frequencies, consistent with a conductive hearing impairment. The latency of the peaks in the ABR waveform were longer in DS than WT mice when compared at the same intensity; however, the latency delays disappeared when the data were compared at the same intensity above thresholds to compensate for the conductive hearing loss. Future studies using wideband tympanometry and absorbance together with detailed histological analysis of the middle ear could illuminate the nature of the conductive hearing impairment in DS mice.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.06

Topic: A.07. Developmental Disorders

Support: NIH R01 AG055581
NIH R01 AG056622

Title: Genetic reduction of eEF2K ameliorates memory deficits and rescues synaptic failure in a mouse model of Down syndrome

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Abstract: Down syndrome (DS) is one of the commonest genetic diseases. Patients with DS usually develop Alzheimer's disease (AD) in their 40s, which will exacerbate their cognitive dysfunction. Previous studies have shown that synaptic failure is a common character of both AD and DS, while *de novo* protein synthesis is vital for synaptic plasticity, leading to the hypothesis that impaired *de novo* protein synthesis might contribute to the pathogenesis of DS. Eukaryotic elongation factor 2 (eEF2) is a part of the ribosome machinery and facilitates transfer of tRNA from A site to P site, thus plays important role in *de novo* protein synthesis. eEF2 can be phosphorylated and deactivated by the only known kinase, eukaryotic elongation factor 2 kinase (eEF2K), which will impair *de novo* protein synthesis. Here we explored the role of eEF2 in cognitive dysfunction of DS. We first checked whether eEF2 was hyperphosphorylated in the brain of Down syndrome patients and we found that phosphorylation of eEF2 was indeed increased in DS patients compaired to controls. The increase of eEF2 phosphorylation in DS patients was also recapitulated in the Ts65Dn mouse model of DS. To further explore the role of eEF2 in DS, we crossed eEF2K+/- mice to Ts65Dn mice, generating four genotypes: WT, eEF2K+/-, Ts65Dn, and Ts65Dn; eEF2K+/-. We found that memory deficits were ameliorated in Ts65Dn; eEF2K+/- mice compared to Ts65Dn mice as assessed by Novel Object Recognition and Morris Water Maze tests. Electrophysiological study showed that synaptic failure (LTP) in Ts65Dn mice was rescued by knocking down eEF2K. These data suggest that genetic reduction of eEF2K can ameliorate memory deficits and rescue synaptic failure in a mouse model of Down syndrome, which provides a potential pharmaceutical target for the treatment of cognitive dysfunction in DS.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.07

Topic: A.07. Developmental Disorders

Support: NIH Grant R01HD100607-01A1

Title: The Kcnj6 gene dose affects early postnatal development: Implications for Down syndrome

Authors: A. TAN, B. LE, R. ZAYTER, D. CHAN, J. JIN, N. MOY, ***A. M. KLESCHEVNIKOV**;
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Abstract: The *Kcnj6* gene, located on human chromosome 21 (HSA21), encodes Girk2 (Kir3.2) subunits of inwardly rectifying potassium channels, which serve as the main effectors of several postsynaptic metabotropic receptors (GABAB, 5HT1A, m2, A1, etc) and play an important role in the regulation of the resting membrane potential and neuronal excitability. The *Kcnj6* gene has

been implicated in several chromosomal disorders, such as Keppen-Lubinsky syndrome, infantile spasm disorder, and Down syndrome. In spite of a different character of changes in the Girk2 channel efficiency (loss of potassium selectivity in the Keppen-Lubinsky syndrome *vs*. an enhancement of otherwise normal signaling in Down syndrome), all *Kcnj6*-related disorders are characterized by developmental and cognitive abnormalities. The mechanisms of the Girk2 channel involvement in these abnormalities are not yet fully understood. Girk2 is expressed at a high level in the early postnatal period in mice and, thus, altered signaling through the Girk2 channels could affect the formation of nascent neural circuits thus leading to abnormal development of the brain. To assess the role of *Kcnj6*/Girk2 in the development of a mouse brain, we used a battery of biochemical, electrophysiological, and behavioral tests to examine the effects of the *Kcnj6* gene dose on the achievement of various developmental milestones. Girk2 expression levels strictly followed the *Kcnj6* gene dose. For example, in the neocortex of p8 pups, the expression levels of Girk2 were: 100 ± 11.0% in Girk2+/+, 46.7 ± 12.1 % in Girk2 +/-, and -0.7 ± 0.8% in Girk2 -/- littermates). Developmental parameters of the Girk2 +/- heterozygous pups were indistinguishable from the littermate Girk2 +/+ controls. On the other hand, Girk2-/- mice showed significant delays in achieving developmental milestones in some of the neonatal behavioral tests (e.g., 'righting' reflex), but not in some other (e.g., "grasping" and "bar holding" reflexes). Thus, similar to the mouse genetic models of Down syndrome which have 3 functional copies of *Kcnj6, Kcnj6* KO mice showed a delay in the early postnatal development. The results suggest that optimal levels of the Girk2 channel signaling are required for normal development of the brain and that either an increase or a significant decrease in Girk2 channel efficiency leads to developmental abnormalities, which could be a basis for cognitive impairment later in life.

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Poster

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Topic: A.07. Developmental Disorders

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Brightfocus BFF17_0008

Title: Epigenomic-wide association study reveals DNA methylation changes in the brains of people with Down syndrome and Alzheimer's disease

Authors: *M. HUENTELMAN[1], I. S. PIRAS[1], S. BERES[1], S. HUDSON[1], S. WRIGHT[2], E. HEAD[2], R. VELAZQUEZ, Jr.[3];
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Abstract: Down syndrome (DS) affects one of every 700 births in the United States. By the age of 40, virtually all people with DS have sufficient beta-amyloid deposits and tau tangles for a neuropathological diagnosis of Alzheimer's disease (AD). The epigenetic changes that occur in key affected brain regions in people with DS and AD (DS-AD) remain elusive. We conducted an Epigenomic-Wide Association Study (EWAS) on post-mortem brain samples from donors with AD (age: 86.7 ± 8.7, M/F ratio: 1.1), DS-AD (age: 74.7 ± 16.7; M/F ratio: 1.2), and controls (CTL; age: 88.1 ± 3.8; M/F ratio: 0.8) across three brain regions: basal forebrain (BF), middle temporal visual area (MT) and middle frontal area (MF). DNA samples were characterized on the Illumina *MethylationEPIC* array. Analysis was conducted using *R-minfi*, and after quality control, we obtained a dataset comprising 191 samples and 757,491 CpG sites. Differential DNA methylation analysis was conducted with *R-limma* after adjusting for confounding factors. The comparison of DS-AD and CTL samples resulted in the detection of 151 statistically significant unique CpG sites across all three brain regions with 13 sites shared between at least two brain regions (all hypermethylated in DS-AD and located within nine different genes including *OTOG* and *SLC26A5*). *OTOG* and *SLC26A5* are associated with hearing loss (adj-p = 0.027). When comparing DS-AD vs AD, we detected 238 unique differentially-methylated CpG sites across the three brain regions with 30 of these sites shared across at least two regions and located in 25 genes, three of which are related to insulin resistance and transport (*AKT1*, *PRKCZ*, and *TBC1D4*; adj-p = 7.6 E-03). Finally, we did not detect any significant changes when comparing AD vs CTL samples. We identified epigenetic changes in genes associated with hearing loss in DS-AD, a common clinical characteristic observed in DS. *OTOG* encodes a protein present in the acellular membranes covering the sensory epithelial patches of the inner ear, and it is associated with autosomal recessive deafness. *SLC26A5* encodes for the motor protein of cochlear outer hair cells, and it is provisionally associated with autosomal recessive deafness as well. Additionally, insulin resistance and signaling changes in DNA methylation seem to distinguish DS-AD vs AD. Metabolic diseases associated with defects of insulin and insulin-related pathways are frequently observed in DS. In conclusion, our results highlight epigenetic changes in CpG sites located in genes involved in two important clinical characteristics of DS: hearing loss and insulin metabolism alterations. This finding will help characterize the molecular determinants of DS and DS-AD.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.09

Topic: A.07. Developmental Disorders

Support: SFARI Grant 702556

Title: Identifying neuronal populations driving behavioral phenotypes in a mouse model of Angelman syndrome

Authors: *N. RINGELBERG, R. REEVES, B. D. PHILPOT;
Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Angelman syndrome (AS) is a severe neurodevelopmental disorder characterized by penetrant seizures, intellectual disability, motor impairment, sleep disruption, and absence of speech. AS is caused by loss of the maternal allele of *UBE3A*, a gene on chromosome 15 that is only expressed from the maternal allele in neurons. While many preclinical studies have aimed to develop therapies to reinstate *UBE3A*, little is known about the neural circuit mechanisms underlying symptomatology in individuals with AS. This has important clinical relevance, as information regarding key cell types driving AS symptoms could inform potential strategies for therapeutic delivery. To determine which neuronal cell types are most important for AS-relevant behaviors, we generated conditional *Ube3a* knock-out mice by crossing female mice harboring a floxed *Ube3a* allele with male *Gad2-cre* and *Vglut2-cre* mice, thereby deleting *Ube3a* from large populations of inhibitory or excitatory neurons, respectively. These mice, along with littermate controls, were then subjected to a battery of behavioral tests that has been previously characterized in AS model mice, including the open field, marble burying, rotarod, and nest building assays. While *Gad2-cre/Ube3a-flox* mice have previously shown enhanced seizure susceptibility, and phenocopy the enhanced delta rhythm of AS mice measured by EEG, these mice demonstrated overt behavioral deficits only in the nest building task. *Vglut2-cre/Ube3a-flox* mice, however, demonstrated deficits on multiple behavioral tasks, while demonstrating comparable nest building to their wild type littermates. These results suggest that the variety of behaviors characteristic of AS model mice are driven by divergent populations of neurons. Furthermore, while GABAergic neuron expression of UBE3A is critical for seizure resilience, this neuronal population appears less important for motor and certain innate behaviors of these mice. These results could provide a framework to further dissect neural circuit mechanisms of AS symptoms, potentially inspiring future disease-modifying treatments.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.10

Topic: A.07. Developmental Disorders

Support: NIH grant MH065635
Angelman Syndrome Therapeutics (FAST)-Italia

Title: Altered protein metabolism in the hippocampus underlies memory impairment of Angelman syndrome mice

Authors: *F. ARIA[1], K. R. PANDEY[3], C. M. ALBERINI[2];
[1]New York Univ., New York City, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY; [3]Ctr. for Neural Sci., New York Univ. Ctr. For Neural Sci., NEW YORK, NY

Abstract: Angelman syndrome (AS) is a genetic neurodevelopmental disorder due to alterations of the 15q11-13 chromosome region, which includes the gene encoding ubiquitin protein ligase E3 (ube3a) and in neurons is paternally imprinted. AS is characterized by intellectual disability, speech and motor defects, sleep problems, and seizures. The mechanisms by which the genetic defects result in AS symptoms are still poorly understood. Using a mouse model of AS based on the maternal deletion of ube3a, we found that the dorsal hippocampus (dHC), a brain region important for memory and cognition, exhibits increased rate of *de novo* protein synthesis measured by surface sensing of translation (SUnSET) and impaired autophagic flux measured by an AAV-mCherry-LC3B viral reporter. Furthermore, the dHC of AS mice show a significant accumulation of proteins critical for brain plasticity, including the immediate early genes (IEGs) ARC, FOS and EGR1, and proteins involved in autophagy such as MLP3B, SQSTM1 and LAMP1. We found that contextual fear conditioning (CFC), as expected, significantly increases hippocampal levels of IEGs, autophagy proteins as well as of autophagic flux in normal mice, but fails to evoke any changes in the dHC of AS mice, where IEGs and autophagy proteins remain accumulated and the autophagic flux is stalled. Treatment with TAT-Beclin 1, a compound that promotes autophagy, rescues CFC memory impairment in AS mice and enhances memory in wild-type mice. These results suggest that a dysregulation in protein metabolism is an important biological deficit of AS brain.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.11

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI 18H02777
JSPS KAKENHI 21H02661

Title: Therapeutic effects of bumetanide on neurological dysfunction in a mouse model of Angelman syndrome

Authors: *K. EGAWA[1], M. WATANABE[2], H. SHIRAISHI[1], A. FUKUDA[3];
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Abstract: Angelman syndrome is a neurodevelopmental disorder caused by the loss of function of the maternally expressed gene *UBE3A*. The main clinical features consist of cognitive dysfunction, epilepsy, motor dysfunction, and behavioral abnormalities. Clinical symptoms overlap with those of autism spectrum disorders, including speech impairment and repetitive behavior. Recently, bumetanide, a loop diuretic, has been proposed as an effective compound for treating autism spectrum disorders by inhibiting the neuronal Na^+-K^+-Cl^- cotransporter 1 (NKCC1), which facilitates Cl^- influx. To investigate the therapeutic potential of bumetanide in Angelman syndrome, we analyzed Cl^- hemostasis and the effects of bumetanide administration a mouse model of Angelman syndrome that lacks the maternal copy of the *Ube3a* gene (*Ube3a*$^{m-/p+}$). We found increased NKCC1 expression at the protein level in the hippocampus of *Ube3a*$^{m-/p+}$ mice. The steady-state intracellular Cl^- concentration ($[Cl^-]_i$) of CA1 pyramidal neurons was not significantly different on average in these animals, but it demonstrated more variance in *Ube3a*$^{m-/p+}$ mice. As a possible mechanism counterbalancing the $[Cl^-]_i$ increases due to NKCC1 activation, we demonstrated that tonic $GABA_A$ receptor-mediated Cl^- influx was significantly reduced in CA1 pyramidal neurons. Chronic administration of bumetanide restored cognitive dysfunction in *Ube3a*$^{m-/p+}$ mice. Seizure susceptibility was also reduced by bumetanide in both *Ube3a*$^{m-/p+}$ and littermate control mice. These results suggest that $[Cl^-]_i$ homeostasis is altered by multiple mechanisms, and aberrantly activated NKCC1 transporters have a pathophysiological impact leading to cognitive dysfunction in *Ube3a*$^{m-/p+}$ mice. Bumetanide administration might be effective for improving cognitive function and epilepsy in patients with Angelman syndrome.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: 605.12

Topic: A.07. Developmental Disorders

Title: Obesity and gender differences induced by ketogenic diet in mice model of Angelman syndrome

Authors: *T. GOTO, S. KIMURA, S. NAKAKUBO, H. SHIRAISHI, A. MANABE, K. EGAWA;
Pediatrics, Hokkaido Univ., Sapporo, Japan

Abstract: Angelman syndrome (AS) is a congenital neurodevelopmental disorder caused by a functional deletion of a maternally expressed gene *UBE3A*. Clinical feature is characterized by

severe mental retardation, epilepsy and movement disorder. Further, female AS individuals are known to present high risk for obesity during adult age. However, its mechanism is largely under elucidated due to lack of the proper model. To investigate possible malfunction of fat metabolism and its sex difference in AS, we analyzed the effects of the long-term high-fat low-carbohydrate diet, also known as ketogenic diet (KD), in mice model of AS which lacks maternally inherited Ube3a gene. 4 to 5-week-old male or female of wild-type (WT) or AS mice were initiated to KD or control chow (Ctrl) ad libitum. Body weight and food intake were evaluated every week. As a result, the weight gain of the AS group ingested KD (AS-KD) was higher than AS group ingested Ctrl or WT group ingested KD. In particular, the female AS-KD showed a significant weight gain from the early period in compared to male AS-KD. Three-way ANOVA of body weight revealed the significant main effects in all factors of gender ($P = 0.0111$), genotype ($P < 0.0001$) and diet ($P < 0.0001$). The secondary interaction was also significant ($P = 0.0013$). Although caloric intake was significantly higher in the KD group ($P = 0.0030$), there was no difference in gender ($P = 0.9533$) or genotype ($P = 0.7801$). The total distance traveled in the open field test was significantly longer in the WT group ($P < 0.0001$), but there was no difference in gender ($P = 0.0733$) and diet ($P = 0.0604$). These results indicate that the obesity shown in female AS-KD was not dominantly caused by calorie over-intake or hypokinesia . Because the imprinting effect of *Ube3a* is restricted to neurons, deficiency of *Ube3a* expression is limited to the central nervous system in AS mice. Thus, a part of central nervous system should be responsible for obesity in AS-KD and its sex difference. In particular, we are now focusing on the hypothalamus which is highly associated with fat metabolism by regulating endocrine and autonomic nervous systems. We will proceed with endocrinological assay from the serum, histological analysis of brown adipocytes, and verification of functional activity in the hypothalamus region. In this study, we illustrated that long-term administration of KD is an appropriate method for creating mice model of obesity in AS. Further analysis will hopefully contribute to elucidating its mechanism and prevention of obesity in adult AS patients.

Disclosures: T. Goto: None. **S. Kimura:** None. **S. Nakakubo:** None. **H. Shiraishi:** None. **A. Manabe:** None. **K. Egawa:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.13

Topic: A.07. Developmental Disorders

Support: Angelman Syndrome Foundation Award #66692

Title: Identifying Protein Targets of the Angelman Syndrome-Linked Ubiquitin Ligase, UBE3A

Authors: *L. DREBUSHENKO, Q. ZHOU, M. DOUGHTY;
Uniformed Services Univ. of Hlth. Sci. Grad. Program In Neurosci., Bethesda, MD

Abstract: Angelman syndrome (AS) is a neurodevelopment disorder characterized by cognitive and language impairments, treatment-resistant seizures, reduced or fragmented sleep, motor ataxia, and a characteristic happy affect. Despite the debilitating features of the disorder, no disease-modifying treatments are currently available. AS arises due to the loss of function of the HECT E3 ligase UBE3A in central nervous system neurons. UBE3A catalyzes the addition of lysine 48 (K48)-linked polyubiquitin chains to substrate proteins, thus targeting those proteins for degradation by the ubiquitin proteasome system (UPS). We hypothesize that loss of UBE3A function results in disrupted proteostasis in our human cortical neuron model, leading to abnormal accumulation of UBE3A substrates. Using a dual SMAD inhibition protocol to derive cortical neurons from human induced pluripotent stem cells, we compared the proteome of neurons derived from a CRISPR-Cas9 gene-edited *UBE3A* knockout (KO) line and its isogenic control. Analysis of the lysates from these neurons by liquid chromatography tandem mass spectrometry (LC-MS/MS) identified 645 proteins of significantly different abundance in the KO versus control, including 332 proteins that were upregulated in the KO. As a possible indicator of their disrupted UBE3A-mediated degradation, we focused on proteins significantly ($p < 0.05$) upregulated by 1.2-fold or higher in the KO. These criteria included 68 protein hits, 40 of which are involved in receptor trafficking, vesicle exocytosis, or neurotransmitter synthesis. Hits included PACSIN1 and GRIPAP1, two proteins with known functions in AMPA receptor endocytosis and recycling, as well as catechol O-methyltransferase (COMT), a key enzyme in the metabolism of catecholamine neurotransmitters, including dopamine. We demonstrate by recombinant protein assays that wild-type but not ligase-dead UBE3A increases K48 polyubiquitination of PACSIN1, GRIPAP1, and COMT. We are currently examining how this ligase-dead mutation in UBE3A affects the rate of degradation of PACSIN1, GRIPAP1, and COMT. By identifying substrates of UBE3A, we offer targets for disease-modifying treatments of AS.

Disclosures: L. Drebushenko: None. **Q. Zhou:** None. **M. Doughty:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.14

Topic: A.07. Developmental Disorders

Support: EHU-UPV PIFBUR17 PhD Grant

Title: role of usp9x in regulating prefrontal cortex excitability and its potential implications for angelman syndrome

Authors: *U. ALDUNTZIN[1,2], N. ELU[2], M. GINGER[1], U. MAYOR[2], A. FRICK[1];
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[2]Dept. of Biochem. and Mol. Biology, Fac. of Sci. and Technol., EHU-UPV, Leioa, Spain

Abstract: Aims: The lack of functional ubiquitin E3 ligase UBE3A in the brain leads to the rare neurodevelopmental disorder Angelman Syndrome (AS), associated with alterations in prefrontal cortex (PFC) function and cognitive impairment. Protein ubiquitination, however, is not only modulated by E3 ligases, but also by deubiquitinating (DUB) enzymes. Identifying and characterizing DUBs responsible of counteracting UBE3A could lead to therapeutic targets that could ameliorate AS symptoms. A previous study from our lab demonstrated that USP9X -a DUB associated with X-linked intellectual disability- counteracts UBE3A mediated ubiquitination. We aim to characterize the role of USP9X in regulating cortical excitability, found to be altered in AS mouse models, and shed light on the potential therapeutic value of USP9X inhibitors. **Methods**: We performed *in vitro* patch clamp recordings in cortical slices of C57BL/6 mice focusing on pyramidal cells of layer V in the medial PFC (mPFC), one of the main output neuron types of the PFC. USP9X activity was blocked using the two specific inhibitors. **Results**: Blocking USP9X led to changes in several parameters of neuronal excitability, the most prominent being an increase in the sag ratio and in the medium after hyperpolarization (mAHP) of mPFC layer V pyramidal neurons. **Conclusions**: Our results suggest that UPS9X regulates the activity or expression of different ion channels that contribute to intrinsic properties regulating basic membrane properties and synaptic integration. Following these results, we will examine whether blocking USP9X activity ameliorates changes observed in AS mouse models, to further investigate the therapeutic potential of USP9X inhibitors.

Disclosures: U. Alduntzin: None. **N. Elu:** None. **M. Ginger:** None. **U. Mayor:** None. **A. Frick:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.15

Topic: A.07. Developmental Disorders

Support: Angelman Syndrome Foundation
NICHD R01HD093771
NIMH R01MH120229
NIH-NINDS P30 NS045892
NIH-NICHD U54 HD079124

Title: Regional and cellular organization of the autism-associated protein UBE3A and its antisense transcript in the brain of the developing rhesus monkey

Authors: *S. SALVADOR[1], C. GONZALEZ RAMIREZ[1], S. CLARK[1], L. JAMES[1], D. G. AMARAL[2], A. C. BURETTE[1], B. D. PHILPOT[1];
[1]Cell Biol. and Physiol., UNC-CH, Chapel Hill, NC; [2]Univ. of California Davis, Sacramento, CA

Abstract: Angelman Syndrome (AS), a rare neuro-genetic disorder, is caused by a mutation or deletions of the maternally-inherited ubiquitin-protein ligase E3A (UBE3A) allele. In mature neurons, the paternal allele of UBE3A is silenced by a noncoding antisense transcript (UBE3A-ATS). The loss of neuronal UBE3A results in severe lifelong symptoms, including intellectual delay, speech impairments, and seizures. Promising gene therapy approaches are being developed which rely either on gene addition of UBE3A or knockdown of the UBE3A-ATS to reinstate expression from the intact paternal UBE3A allele. Safe delivery of these therapeutics requires accurately knowing when, where, and in which cell types UBE3A and UBE3A-ATS are expressed in the developing human brain. We know very little about UBE3A and UBE3A-ATS distribution in the human brain, but it is generally assumed that they closely mirror that of the rodent. This lack of knowledge may lead to inappropriate delivery and treatment strategies for AS, if the assumption is false. To start addressing this issue, we examined the spatiotemporal expression of UBE3A and UBE3A-ATS in the rhesus macaque monkey (Macaca mulatta), a close relative to humans.

Combining high-resolution immunohistochemistry with hybridization chain reaction in situ, we mapped UBE3A and UBE3A-ATS regional and cellular expression in normal prenatal (gestational day 50, 100, and 150), neonatal (2 and 4 weeks old), and adult (3 months and 5 years) macaque brains. We find that UBE3A is expressed in most cells in the developing brain. Between GD50 and GD150, UBE3A undergoes a shift in its subcellular localization, becoming progressively more nuclear. Interestingly, this shift closely matches the onset of UBE3A-ATS expression. This is striking in the neocortex at GD 100: in the superficial layers, UBE3A staining is predominantly extra-nuclear with very little UBE3A-ATS detectable, whereas in the deeper, more mature layers, UBE3A staining is more nuclear and UBE3A-ATS is detected in most neuronal nuclei. Postnatally, UBE3A is expressed in most cells, but different cell types express different levels of UBE3A. For example, in the mature neocortex, neurons have higher levels of nuclear UBE3A than glial cells. We also find that most if not all cells expressing UBE3A-ATS are neurons.

Our findings about UBE3A expression in the developing primate brain will inform gene therapy and reinstatement strategies to treat AS.

Disclosures: S. Salvador: None. **C. Gonzalez Ramirez:** None. **S. Clark:** None. **L. James:** None. **D.G. Amaral:** None. **A.C. Burette:** None. **B.D. Philpot:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.16

Topic: A.07. Developmental Disorders

Support: Angelini Grant 168(A)MD21320

Title: Involvement of Prader-Willi syndrome: bumetanide restores cognition in Snord116-deleted mice

Authors: *M. CHELLALI[1,2], M. BOLLA[1], A. MELLONI[1,3], G. COMO[1,4], L. CANCEDDA[1], V. TUCCI[1];
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Abstract: Prader Willi syndrome (PWS) is a neurodevelopmental disorder, caused by the loss of some paternal genetic inheritance linked to chromosome 15 (15q11-q13). The hallmark of PWS is excessive eating. However, other health issues such as intellectual disability, social impairment, compulsive behavior and sleep disturbance negatively affect the lives of PWS people and their families. In recent years, defective Cl^- homeostasis in neurons has been linked to an increasing number of brain disorders including neurodevelopmental disorders. Interestingly, these brain disorders have very different aetiologies, but they all share common symptoms, such as intellectual disability and/or social impairment, compulsive behavior and sleep disturbance. Interestingly, for all these health issues GABAergic transmission through Cl^- permeable $GABA_A$ receptors plays a critical role. In particular, pharmacological inhibition of the Cl^- importer NKCC1 rescues core symptoms in mouse models and people suffering from many of these brain disorders. However, whether Cl^- homeostasis is deregulated and responsible for social impairment and intellectual disability in PWS is currently unknown. Here, we observed that the *Snord116* mutant mouse model of PWS shows an increased expression of NKCC1 specifically in the hippocampus. Interestingly, we observed a dysregulation of Cl transporters also in human hippocampus from post-mortem PWS brain samples. PWS Chronic systemic treatment with bumetanide (an FDA-approved NKCC1 inhibitor) rescued cognitive deficits during hippocampal-dependent cognitive tests in adult PWS mice. Of note, *Snord116* mutant mice showed no deficits in social behaviors. Thus, our results indicate that *Snord116* mutant mice are a valuable model to study cognitive impairment in PWS and indicate a key role for Cl^- homeostasis in their cognitive performance. Moreover, our study proposes a target and a new potential pharmacological approach in PWS.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.17

Topic: A.07. Developmental Disorders

Support: NIMH Diversity Supplement Award (Grant R01MH120133-04)

Title: Cognitive and motivational consequences of knocking-out the snord116 paternal allele in catecholaminergic cells

Authors: *L. S. VASQUEZ[1,2,3], S. M. STACK[3,2], W. W. TAYLOR[1,2,3], B. G. DIAS[4,2,3];
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Abstract: Prader Willi Syndrome (PWS) is a rare genetic disorder that is primarily caused by a loss of genes within a critical region on the paternal allele of chromosome 15. Phenotypically, PWS presents with perturbations of cognition and motivation (Adhikari et al., 2019; Bervini and Herzog, 2013). Of the paternal alleles deleted on chromosome 15, whole brain knockouts of the *snord116* paternal allele gene cluster in mice results in PWS phenotypes similar to humans (Ding et al., 2008; Bieth et al., 2015; Miller et al., 2011). Missing from such analyses are the effects of deleting the paternal *snord116* allele in specific neuronal populations. This gap prevents us from discovering and developing pharmacotherapeutics that could alleviate specific PWS-associated endo-phenotypes. With catecholamines implicated in both cognitive and motivational behavior, we used a CRE-loxP approach to determine the consequences of deleting the paternal allele of *snord116* in catecholaminergic cells on cognition and motivation. Experimental CRE animals had the paternal allele of *snord116* deleted in catecholaminergic cells, while control animals had this allele intact. Auditory fear conditioning and extinction training was performed to investigate learning and memory. A progressive ratio schedule of reinforcement (PR) was used to assay motivation. Cognitive changes were seen in CRE animals during recall of extinction training. Motivation was not impacted in CRE animals as measured by breakpoint ratio in the PR test. Our data suggest that deletion of the paternal *snord116* allele in catecholaminergic cells results in altered learning and memory. This approach has the potential to shed light on the neurobiology underlying cognitive deficits associated with PWS.

Disclosures: L.S. Vasquez: None. **S.M. Stack:** None. **W.W. Taylor:** None. **B.G. Dias:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.18

Topic: A.07. Developmental Disorders

Support: NIH R01NS114086

Title: Characterizing Sleep Behavior in Pitt-Hopkins Model Mice

Authors: *L. M. JAMES, S. S. MOY, B. D. PHILPOT;
UNC Chapel Hill, Chapel Hill, NC

Abstract: Pitt-Hopkins syndrome (PTHS), a severe neurodevelopmental disorder caused by monoallelic deletion or loss-of-function mutations of the *TCF4* gene, is defined by intellectual disability, lack of speech, motor delay, microcephaly, and seizures. Additionally, individuals with PTHS experience breathing disruption, the most common pattern being a period of very fast

breathing followed by apnea. While breathing irregularities are thought to occur only while awake, individuals with PTHS also suffer from sleep disruption. It is unclear whether sleep disturbance in PTHS is due to breathing irregularities or impaired circadian circuitry. Studies to characterize sleep disturbance in PTHS are limited but suggest that it is common. A recent study of 22 individuals with PTHS highlighted that sleep disturbances such as difficulty falling asleep or sleeping through the night were very common (55%). Those with sleep disruption included individuals with full or partial gene deletions, and frameshift or missense variants. Another study of 101 individuals with PTHS also reported that sleep disruptions are common. Sleep disturbances can be highly disruptive to development, growth, and daytime performance and also perturb care-taker well-being. For these reasons, sleep disruption is a highly-relevant behavior to measure and consider in studies of therapeutics for neurodevelopmental disorders, including PTHS. Using the PTHS mouse model generated by our lab ($TCF4^{STOP/+}$), we will investigate whether PTHS model mice exhibit sleep disruption at baseline, and whether they are more sensitive to sleep disturbance. PTHS model mice and wild-type littermates will be evaluated for sleep patterns across 7 days under a 12/12 hour light/dark cycle. Mice will be placed in individual cages, each set on a pressure-sensitive detector pad (PiezoSleep system Signal Solutions). Baseline time sleeping, average number of sleep bouts, and average length of sleep bouts will be measured during the morning, afternoon, early night, and late night of days 1-3. During the morning of day 4, sleep will be disturbed for a 3-hour period by placing mice into a series of settings such as open field chambers, acoustic startle tests, and activity wheels. After the 3-hour sleep disruption, sleep rebound will be evaluated across 3 additional days to determine recovery time of regular sleep patterns. Our ongoing study will determine whether PTHS model mice exhibit sleep disruption at baseline or are more sensitive to sleep disturbance. Our study aims to potentially add a highly relevant and translatable behavioral measure to our preclinical toolbox for testing Pitt-Hopkins syndrome therapeutics.

Disclosures: L.M. James: None. **S.S. Moy:** None. **B.D. Philpot:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.19

Topic: A.07. Developmental Disorders

Support: State of California 2021-2022 Budget, SB 129 #44 (Jordan's Guardian Angels)

Title: Characterization of dopaminergic dysfunction in murine models of Jordan's syndrome.

Authors: *I. J. VILLEGAS[1], D. L. CAMERON[1], C. JONG[2], C. CHEN[2], R. A. MERRILL[2], S. STRACK[2], K. D. FINK[1];
[1]Ctr. for Interventional Genetics, MIND Institute, Stem Cell Program and Gene Therapy Center, Inst. for Regenerative Cures and Dept. of Neurology, Univ. of California Davis Hlth. Systems,

Sacramento, CA; [2]Dept. of Neurosci. and Pharmacology, and Iowa Neurosci. Institute, Univ. of Iowa, Iowa City, IA

Abstract: Intellectual disability (ID) affects nearly 1% - 3% of the population worldwide and can range from mild to profound in severity. In severe cases of ID, causality is commonly linked with an underlying genetic defect. PPP2R5D-related ID disorder, also known as Jordan's syndrome (JS), is a rare monogenic form of ID that is characterized by moderate to severe ID and neurodevelopmental delay, in conjunction with other comorbidities including hypotonia, macrocephaly, seizures, and autism spectrum disorder. JS is caused by de novo missense mutations that occur within *PPP2R5D*. *PPP2R5D* encodes an isoform of the regulatory B subunit, B56δ, for the protein phosphatase 2A (PP2A) holoenzyme. PP2A is a major serine/threonine phosphatase that regulates phosphorylation of various substrates across many cell signaling pathways. PPP2R5D is enriched within the CNS and plays a vital role to negatively regulate cell growth and proliferation. Previous research has shown that mutations within *PPP2R5D* can affect substrate specificity, subcellular localization, and the catalytic activity of PP2A-B56δ. Furthermore, recent clinical case reports have identified five JS patients concurrently diagnosed with early-onset parkinsonism. In these reports, patients were described as levodopa-responsive indicating dopamine dysfunction in association with *PPP2R5D* mutations. To further investigate this association, we utilized novel murine models of JS containing the equivalent human pathogenic mutations in *Ppp2r5d* and examined the pathophysiological effects of *Ppp2r5d* mutations in brain tissue. In our study, we observed significantly reduced tyrosine hydroxylase (TH) immunoreactivity within the substantia nigra of JS mice. Additionally, through immunohistochemistry and immunoblotting, we found significant changes in phosphorylation of TH and dopamine-and cAMP-regulated phosphoprotein (Darpp32), both direct substrates of PP2A-B56δ, within the substantia nigra and striatum, and whole brain, respectively. Moreover, utilizing PRIME editing we generated mutations within Exon 5 in Neuro2a cells and examined the molecular pathophysiology of *Ppp2r5d* mutations in these differentiated dopaminergic cells. In summary, our group is the first to report dopaminergic abnormalities within the substantia nigra and striatum of JS mice. These data support previous clinical case reports of dopamine dysfunction in association with *PPP2R5D* mutations and warrant further investigation.

Disclosures: I.J. Villegas: None. **D.L. Cameron:** None. **C. Jong:** None. **C. Chen:** None. **R.A. Merrill:** None. **S. Strack:** None. **K.D. Fink:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.20

Topic: A.07. Developmental Disorders

Support: NSERC Grant 103328

Title: Regulation of Borjeson-Forssman-Lehmann Syndrome by a novel PHF6/EphR transcriptional pathway

Authors: *D. RASOOL[1,2], A. BURBAN[1], A. SHARANAK[1], V. SOLEIMANI[1], A. BONNI[3], H. NAJAFABADI[1], A. JAHANI-ASL[1,2];
[1]McGill Univ., Montreal, QC, Canada; [2]Univ. of Ottawa, Ottawa, ON, Canada; [3]Hoffmann-La Roche, Basel, Switzerland

Abstract: PHD Finger Protein 6 (PHF6) is a transcriptional regulator with its germline mutations causing the X-linked intellectual disability Börjeson-Forssman-Lehmann Syndrome (BFLS), a congenital neurodevelopmental disorder. The precise mechanisms by which PHF6 regulates transcription, and its mutation causing BFLS and cognition deficits remain poorly understood. Here, we employed next generation sequencing platforms, including ChIP-Seq and RNA-Seq, to gain mechanistic insights into PHF6 function. We identified 2473 PHF6 binding sites, with regions significantly overlapping $(CA)_n$-microsatellite repeats enriched near genes involved in developmental processes, including central nervous system development and neurogenesis. Through intersection of ChIP-Seq and RNA-Seq data, we found that PHF6 binding to the TSS inhibits Pol II recruitment and inhibits expression, whereas PHF6 binding further downstream of the TSS increases expression. Importantly, we identified a large panel of Ephrin receptors (EphR) as direct target genes of PHF6. Through luciferase reporter assay and ChIP-qPCR, we found that PHF6 directly occupies the promotors of EphR genes. Additionally, we observed a decrease in EphR expression in BFLS mouse models. Furthermore, we found that mice harboring a BFLS mutation exhibited an increase in embryonic neural stem cell (eNSC) numbers, self-renewal, and increased expression of stem cell markers, Sox2 and Nestin. In conclusion, we established that a novel PHF6/EphR transcriptional pathway regulates neurogenesis, and impairment of this pathway may lead to the BFLS pathogenesis.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.21

Topic: A.07. Developmental Disorders

Support: NIH 5R01NS109304-03
SFARI, award ID 631904

Title: AAV integration into Cas9-induced double-stranded breaks is an extremely common event, with implications for use of AAV as a gene therapy vector in the brain

Authors: *H. BAZICK, H. MAO, J. M. WOLTER, J. K. NIEHAUS, M. J. ZYLKA;
Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Angelman Syndrome (AS), a severe neurodevelopmental disorder caused by disruption of the maternally-inherited ubiquitin protein ligase E3A (*Ube3a*) allele, is characterized by severe sleep dysfunction, seizure disorders, impaired cognition and speech, motor deficits, and microcephaly. Due to epigenetic silencing of the paternal allele of UBE3A (patUBE3A) by a long non-coding antisense RNA (*Ube3a-ATS*) in neurons, mutation or deletion of maternal *Ube3a* results in near complete loss of UBE3A protein in the brain. Our previous study revealed that adeno-associated virus (AAV)-CRISPR/Cas9 gene therapy targeted to 75-repetitive *Snord115* sequences along *Ube3a-ATS* leads to integration of the AAV vector into Cas9 target sites, trapping *Ube3a-ATS*, unsilencing patUBE3A, and alleviating AS phenotypes in mice with a dual embryonic/post-natal day 1 injection. Based on this mechanism of unsilencing, we hypothesized that integration of AAV at a single unique site should sufficiently block *Ube3a-ATS* transcription to unsilence patUBE3A. Thus, we designed 25 guide RNAs (gRNAs) that targeted unique sites within *Ube3a-ATS*. When delivered in an AAV vector along with Cas9 to primary neuron cultures, the best gRNAs individually achieved only 46% knockdown (KD) of *Ube3a-ATS*. This contrasted with 80% KD when a single gRNA was used that targeted 78 *Snord115* genes. However, examination of comprehensive editing profiles following AAV/Cas9 treatment in neuron cultures using modified Anchored Multiplex PCR (AMP)-seq coupled with suppression-PCR and target-enrichment revealed that AAV integration accounts for approximately 80% of all editing events, while insertions/deletions (indels) account for only 20%. Using a reporter assay, we found that the most frequent indels failed to block gene transcription, while integration of AAV-derived elements disrupted downstream gene expression. Our study shows that AAV integration is a frequent outcome of AAV/Cas9 therapy in neurons, suggests that AAV-derived elements are capable of disrupting genes, and AAV integration may be the primary mechanism of *Ube3a-ATS* knockdown in the treatment of AS when using AAV/Cas9 gene therapy. Our work has implications for AAV as a gene therapy vector, as we find that AAV integration into double-stranded breaks is an extremely efficient process in neurons, and that integrated AAV-derived elements can disrupt gene expression.

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Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.22

Topic: A.07. Developmental Disorders

Support: Taysha Gene Therapies, Inc.

Title: Effective shRNA unsilencing of imprinted genes in Angelman and Prader-Willi Syndromes

Authors: *R. BUTLER, H. KANG, V. ZARIC, A. RAHIM, S. DEVRIES, Z. MEMON, S. GRAY;
Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Angelman (AS) and Prader-Willi (PWS) Syndromes are imprinting disorders where the defective gene(s) are naturally expressed on only one allele. AS is a neurodevelopmental disorder caused by the deficiency of functional Ubiquitin Protein Ligase E3A (UBE3A) resulting in intellectual deficits, ataxic gait, lack of speech and frequent laughter. PWS is a neurodevelopmental disorder caused by the loss of function of a series of PWS-related genes on human chromosome 15, including *SNORD116* and *SNRPN*, resulting in weak muscle tone (hypotonia), feeding difficulties, poor growth, and delayed development. *UBE3A* expression is regulated through genetic imprinting whereby the maternal allele is expressed and the paternal allele is silenced in a neuron-specific manner; conversely, PWS-related genes are paternally expressed and maternally silenced. *UBE3A* silencing is induced with a portion of a large, non-coding antisense transcript specific to *UBE3A* (*UBE3A-ATS*) and maternal silencing of PWS genes is induced with euchromatic histone lysine methyltransferase 2 (EHMT2). Previously it has been demonstrated that anti-sense oligonucleotides and CRISPR/Cas9 targeting *UBE3A-ATS* elevated paternal expression of UBE3A and improved behavior deficits in an AS mouse model; small molecule targeting of *EHMT2* elevated maternal expression of *SNORD116* and *SNRPN* and improved survival in a PWS mouse model. In our studies, we tested the hypothesis that shRNA-induced inhibition of the silencers of imprinted genes involved in AS and PWS can induce expression of the silenced and corrected form of the genes. First, we screened siRNA candidates targeting *UBE3A-ATS* and *EHMT2* in a human neuroblastoma cell line (N=3). We discovered several candidates which reduced *UBE3A-ATS* and *EHMT2* while concomitantly increasing *UBE3A* and *PWS genes* transcript levels. We also differentiated induced pluripotent stem cells (iPSC) from AS and PWS patients into neurons and found that *UBE3A-ATS* expression increased at each stage during the development into neurons. Top siRNA candidates were converted into shRNA oligonucleotides and cloned into a self-complementary construct plasmid. We transfected lead candidate shRNA plasmids into iPSC-derived neuronal progenitor lines. Preliminary data based suggests that shRNA plasmid candidates reactivated silenced gene and protein expression in both AS and PWS iPSC neural cell lines. This study demonstrates initial proof-of-concept to use an AAV vector to treat AS and PWS via shRNA-mediated knock-down of genetic silencers. Conceptually, using an AAV-based approach may provide a one-time dosing regimen to confer permanent expression of naturally silenced genes.

Disclosures: **R. Butler:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Taysha Gene Therapies, Inc. **H. Kang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Taysha Gene Therapies, Inc.. **V. Zaric:** None. **A. Rahim:** None. **S. DeVries:** None. **Z. Memon:** None. **S. Gray:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Taysha Gene Therapies, Inc..

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.23

Topic: A.07. Developmental Disorders

Title: Using antisense oligonucleotides to reactivate the paternal UBE3A allele in Angelman Syndrome patient-derived neurons

Authors: *H. SMEENK[1], B. LENDEMEIJER[1], M. G. BUURMA[1], E. MIENTJES[2], C. MILAZZO[2], Y. ELGERSMA[2], F. M. S. DE VRIJ[1], S. A. KUSHNER[1];
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Abstract: Angelman Syndrome (AS) is a severe neurodevelopmental disorder, characterized by developmental delay, behavioral abnormalities, and seizures. Non-homology between human and non-human genomes is a major issue for investigating genetic therapies such as antisense oligonucleotides (AONs), particularly for the widely used AS mouse model. AS is caused by loss-of-function mutations of the maternal *UBE3A* gene, which exhibits parent-of-origin imprinting in mature neurons. The paternal copy of *UBE3A* is silenced through expression of the *UBE3A* Antisense Transcript (*UBE3A-ATS*). Therefore, *UBE3A-ATS* is an interesting potential AS therapeutic target using AON therapy, as targeted degradation of *UBE3A-ATS* reverses paternal imprinting and restores neuronal *UBE3A* expression. However, due to low sequence homology, AONs targeting human *UBE3A-ATS* cannot effectively be tested in mice. To address this problem, we have generated human neuronal cultures from induced pluripotent stem cells (iPSCs) of two siblings carrying a nonsense mutation (W577*) in the *UBE3A* gene. Neurons were generated through overexpression of neurogenin-2 in human iPSCs, which recapitulate *UBE3A* gene imprinting and result in the loss of UBE3A expression in neurons derived from AS patient iPSCs. Here, we show that treatment of AS neurons with an AON against *UBE3A-ATS* reactivates UBE3A expression from the paternal *UBE3A* allele. In addition, we have transplanted AS neurons into the brain of neonatal immunodeficient mice, which integrated and matured in the mouse brain, and exhibited the expected pattern of *UBE3A* imprinting. Moreover, transplanted mice receiving intraventricular injections of the human-specific *UBE3A-ATS* AON successfully reactivated the paternal *UBE3A* allele in transplanted human AS neurons. Together, these data show that paternal UBE3A expression can be efficiently reactivated using AONs, both *in vitro* and *in vivo*, and highlight the therapeutic potential of AONs for the treatment of AS.

Disclosures: H. Smeenk: None. **B. Lendemeijer:** None. **M.G. Buurma:** None. **E. Mientjes:** None. **C. Milazzo:** None. **Y. Elgersma:** None. **F.M.S. De Vrij:** None. **S.A. Kushner:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.24

Topic: A.07. Developmental Disorders

Support: Rett Syndrome Research Trust 21-3753 A21-4017
Loulou Foundation and UPenn Orphan Disease Center CDKL5– 19 – D-104 - 01

Title: An Epigenetic CRISPR/dCas9 Approach to CASK-Related Disorders

Authors: *C. E. GONZALEZ[1,2,3,4,5], J. A. HALMAI[1,2,3,4,5], K. FINK[1,2,3,4,5];
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Abstract: CASK-related disorders are X-linked neurodevelopmental disorders that predominantly affect females. These disorders such as microcephaly with pontine and cerebellar hypoplasia (MICPCH) and CASK-related intellectual disability affect an important regulatory gene in the brain, calcium/calmodulin-dependent serine protein kinase (CASK). They are typically the result of a loss of function mutation, which females are partially protected from due to X-chromosome inactivation (XCI). As females have two X-chromosomes, disease causing mutations in these genes are diluted out by the mosaicism caused by XCI random inactivation, which allows for affected females to survive past birth. Previously, the Fink lab has shown the ability to target *CDKL5*, a X-linked gene associated with CDKL5-deficiency disorder, in human neuron-like cells and reactivate the XCI-silenced healthy allele using our dual effector CRISPR/dCas9 epigenetic approach. Our current goals are to optimize this approach with a smaller split-CRISPR orthologue, sadCas9, to meet the packaging limit of an Adeno-associated Virus (AAV). Additionally, we aim to apply this epigenetic rescue technology to other X-linked intellectual disabilities such as CASK-related disorders, in a patient derived cell model. We aim to accomplish this through cloning and testing our novel construct to target the *CASK* promoter. Expression of full-length epi-sadCas9 will be visualized through Western Blot and optimal targeting efficacy will be assessed through gRNA screens measured via RT-qPCR *CASK* expression. XCI-reactivation will be tested through Next-Generation and bisulfite sequencing. Downstream molecular effects will be measured through RT-qPCR and Western Blot. Results show the ability to target *CASK* and assess gene expression and methylation status as an approach to rescue XCI-silenced WT *CASK*. This project allows for a therapeutic platform that can be packaged in a clinically relevant AAV delivery method and applied to CASK-related disorders.

Disclosures: C.E. Gonzalez: None. **J.A. Halmai:** None. **K. Fink:** None.

Poster

605. Mechanisms of Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 605.25

Topic: A.02. Postnatal Neurogenesis

Support: R01HL139712 (N.I.)
R01HL146670 (N.I.)

Title: The Effects of Tetrahydrobioterin on Structural Connectivity in a Piglet Model of Chronic Hypoxia.

Authors: *V. LAM[1], S. C. TU[2], K. KOBAYASHI[2], J. LI[2], M. AYODEJI[2], A. AGARONYAN[4], S. LIN[4], A. SINHA[3], S. XU[3], P. C. WANG[4], T.-W. TU[4], N. ISHIBASHI[2];
[1]Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC; [2]Ctr. for Neurosci. Res., [3]Children's Natl. Hosp., Washington, DC; [4]Dept. of Radiology, Howard Univ., Washington, DC

Abstract: Cardiac anomalies resulted from congenital heart disease (CHD) are subject to reduction in blood flow and oxygen delivery to the brain in fetus leading to deficits in hyperactive, learning, and cognitive function. Chronic hypoxemia (CHx) piglet models are promisingly used to study neurodevelopment abnormalities in this patient population as patients' brains both reduce in size, white matter volumes, gyrification, and subventricular zone width. In addition, we have previously demonstrated that the level of Tetrahydrobiopterin (BH4) level in CHx postnatal mouse brains was significantly reduced and the treatment of BH4 could mitigate the toxic effects on the developing white matter. Connectome analysis introduces an advanced computation method to achieve sensitive and unbiased evaluation to study comprehensive brain development and a wide range of neurodevelopmental disorders. This study investigates the impact of CHx on the pig brain connectivity and the effects of BH4 supplementation using structural connectome. High-resolution diffusion tensor images were collected from postnatal pig brains in three groups: normoxia (Nx), hypoxia (Hx) and hypoxia treated with BH4 (BH4). An in-house 3D piglet brain atlases with 68 cortical gray matter regions were used to generate connectogram in Mrtrix3 following a series of denoising, registration, fiber reconstruction and connectivity matrix generation. Binary and weighted connectivity matrices were analyzed using a Brain Connectivity Matlab toolbox. Clustering coefficient and path length in piglet brain networks of each group was significantly higher than in random networks averaged from 1000 comparable ones (student's test, $p<0.0001$) suggesting the high capacity of exchanging information. Network efficiency though was preserved in all three piglet groups, they all obtained small-word characteristics ($\sigma >1$), suggesting brain regions are densely connected through relatively few intermediate steps. Control brains achieved higher rich club coefficient than Hx brains ($1.26 > 1.1$, paired t-test, $p<0.01$) with 19 rich club regions comparing to 10 in Hx. Treatment with BH4 partially retrieved rich club regions to 14 as well as fractional anisotropy value at the lost connections in Hx brains. These findings possibly reveal postnatal microstructural alterations in Hx white matter, which will need cellular and microstructural analyses. Structural connectome analysis is a robust and non-invasively method to elucidate hypoxia-induced along with cellular/molecular changes and to establish new therapeutic approach to improve abnormal neurodevelopment in children suffering CHD.

Disclosures: V. Lam: None. **S.C. Tu:** None. **K. Kobayashi:** None. **J. Li:** None. **M. Ayodeji:** None. **A. Agaronyan:** None. **S. Lin:** None. **P.C. Wang:** None. **T. Tu:** None. **N. Ishibashi:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.01

Topic: A.07. Developmental Disorders

Support: NIMHR01MH115005
NIMHR01MH115005-02S1

Title: Role of PSD signaling networks in human models of neurodevelopmental disease

Authors: I. FLORES[1], J. JIANG[1], V. CLEMENTEL[1], N. GRAHAM[2], N. HARTEL[2], *M. COBA[1,3,4];
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Abstract: Synaptic proteins such as Dlgs, Dlgaps, Shanks and Syngap1 are connected in large protein interaction networks at the postsynaptic site of glutamatergic neurons and have been associated to a large number of neurodevelopmental disorders. While their contribution to disease related phenotypes have been well documented in mature rodent synapses, their role in early neuronal development and in non-neuronal cell types is not known. It is not known if these set of proteins are associated in the same protein interaction networks (PINs) in early neuronal development. In particular in human models of neurodevelopmental disease. Here we use the postsynaptic density (PSD) protein TNIK as a model of a classical PSD signaling hub associated to neurodevelopmental disease. Using hiPSC models of disease and TNIK disfunction we show that PSD PINs are dysregulated by TNIK in immature neurons and neuronal progenitor. TNIK impairs neuronal progenitor cells and human immature synapses using different signaling mechanisms and associating to different sets of PSD proteins. These results suggests a widespread role of PSD proteins during early stages of neuronal development and in neuronal progenitor cells that can contribute to different phenotypes of neurodevelopmental disease.

Disclosures: I. Flores: None. **J. Jiang:** None. **V. Clementel:** None. **N. Graham:** None. **N. Hartel:** None. **M. Coba:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.02

Topic: A.07. Developmental Disorders

Support: NIMHR01MH115005
NIMHR01MH115005-02S1

Title: Patient-derived SYNGAP1 mutations disrupts excitatory and inhibitory signaling networks in human models of neurodevelopmental disease

Authors: *I. FLORES[1], J. JIANG[1], B. J. WILKINSON[1], V. CLEMENTEL[2], N. HARTEL[3], N. GRAHAM[3], M. P. COBA[4,1,5];
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Abstract: The synaptic Ras/Rap GTPase-activating protein, SYNGAP1, is one of the most prevalent genes associated with developmental delay and intellectual disability. Consequently, mutations disrupting SYNGAP1 function are now defined as causative for SYNGAP1-related intellectual disability (SYNGAP1-ID). Like many other proteins associated with developmental delay, SYNGAP1 is highly enriched at the post-synaptic density (PSD) of mature glutamatergic synapses and has been established as a major regulator of synaptic function. However, its role in gabaergic neurons and at early stages of neuronal development are not well known. Recent advances in stem cell biology and gene editing have allowed us to start investigating the role of SYNGAP1 mutations found in patients, and their role in synaptic dysfunction at early stages of synaptic and neuronal development. Here, we characterized the role of SYNGAP1 mutations found in patients with ID, in excitatory and inhibitory synaptic signaling networks using human models of neurodevelopmental disease. For this reason we used a combination of CRISPR/Cas9 gene editing technology, patients-derived iPSC neurons, multielectrode arrays (MEAs), Mass spectrometry and spine morphology analysis. We will show the impact of different mutations in SYNGAP1 on synaptic function and how these mutations disregulates synaptic signaling networks in immature synapses from excitatory and inhibitory neurons and their role in neurodevelopmental disease.

Disclosures: I. Flores: None. **J. Jiang:** None. **B.J. Wilkinson:** None. **V. Clementel:** None. **N. Hartel:** None. **N. Graham:** None. **M.P. Coba:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.03

Topic: A.07. Developmental Disorders

Support: NRF-2020R1A5A1019023
NRF-2022R1A2C1004913
KHIDI-HU21C0071
BK21 Four Biomedical Science Program
NRF-2021R1F1A1049169

Title: Functional role of GRM7 mutations in neurodevelopmental disorders

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Abstract: Metabotropic glutamate receptor 7 (mGlu7) is an inhibitory heterotrimeric G-protein-coupled receptor that modulates neurotransmitter release and synaptic plasticity at presynaptic terminals in the mammalian central nervous system. Recent studies have shown that rare mutations in glutamate receptors and synaptic scaffold proteins are associated with neurodevelopmental disorders (NDDs). However, the role of presynaptic mGlu7 in the pathogenesis of NDDs remains largely unknown. Recent whole-exome sequencing (WES) studies in families with NDDs have revealed that several missense mutations (c.1865G.A:p.R622Q; c.461T.C:p.I154T; c.1972C.T:p.R658W and c.2024C.A:p.T675K) or a nonsense mutation (c.1757G.A:p.W586X) in the GRM7 gene may be linked to NDDs. In the present study, we investigated the mechanistic links between GRM7 point mutations and NDD pathology. We find that the pathogenic GRM7 I154T and R658W/T675K mutations lead to the degradation of the mGlu7 protein. In particular, the GRM7 R658W/T675K mutation results in a lack of surface mGlu7 expression in heterologous cells and cultured neurons isolated from male and female rat embryos. We demonstrate that the expression of mGlu7 variants or exposure to mGlu7 antagonists impairs axon outgrowth through the mitogen-activated protein kinase (MAPK)-cAMP-protein kinase A (PKA) signaling pathway during early neuronal development, which subsequently leads to a decrease in the number of presynaptic terminals in mature neurons. Treatment with an mGlu7 agonist restores the pathologic phenotypes caused by mGlu7 I154T but not by mGlu7 R658W/T675K because of its lack of neuronal surface expression. These findings provide evidence that stable neuronal surface expression of mGlu7 is essential for neural development and that mGlu7 is a promising therapeutic target for NDDs.

Disclosures: **J. Song:** None. **Y. Suh:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.04

Topic: A.07. Developmental Disorders

Support: NIH NINDS 5K08NS112598-02

Title: Dysregulation of FMRP Metabolism in Tuberous Sclerosis Complex

Authors: *K. D. WINDEN[1], T. PHAM[2], M. SAHIN[1];
[1]Neurol., [2]Neurobio., Boston Children's Hosp., Boston, MA

Abstract: Autism spectrum disorder (ASD) is a common neurodevelopmental disorder but treatment options and understanding of the pathophysiological processes are limited. Tuberous Sclerosis Complex (TSC) and Fragile X Syndrome (FXS) are two genetic disorders with high prevalence of ASD. TSC is caused by mutations in either *TSC1* or *TSC2*, whereas FXS is caused by silencing of the *FMR1* gene and reduced expression of the Fragile X Messenger Ribonucleoprotein 1 (FMRP). Previously, we found that FMRP target genes were down-regulated in TSC2-deficient neurons, and therefore, we examined the dysregulation of FMRP in these cells. We initially used primary rat neurons with knock-down of *Tsc2* using a short hairpin RNA (sh-*Tsc2*) and treated neurons with cycloheximide, a protein synthesis inhibitor. We found that FMRP was significantly reduced in TSC2-deficient neurons after 8 hours of protein synthesis inhibition, suggesting increased degradation of FMRP. Importantly, this effect was abrogated with proteasome inhibition using MG132. To verify these findings, we used a tagged FMR1 construct under a tetracycline-inducible promoter. *FMR1* expression was induced with doxycycline and then doxycycline was sequentially removed from subsets of neurons. Exogenous FMRP was measured using immunostaining of the epitope tag, and we found that this signal decreased faster in sh-*Tsc2* neurons. We repeated the same experiment using an FMRP sequence with mutations in a known ubiquitination recognition (D-box) and observed reduced rates of FMRP degradation. We also verified the increased degradation of FMRP in cortical neurons derived from human induced pluripotent stem cells (iPSC). We used three isogenic lines derived from a TSC patient (*TSC2*+/-), where the patient mutation was either corrected (*TSC2*+/+) or had a mutation in the second allele (*TSC2*-/-). We created a self-labeling FMRP by fusing it to the HALO enzyme. We treated neurons with a HALO-ligand dye to label FMRP, removed the dye, and assayed fluorescence at subsequent intervals as a measurement of the residual FMRP. We found that TSC2-deficient neurons had increased FMRP degradation compared to the control. We then repeated the experiment with the same mutant FMRP described above and observed no significant difference in FMRP. We also repeated these sets of experiments on a second set of isogenic TSC2 iPSC lines. Taken together, we have found that FMRP degradation was increased in TSC2-deficient neurons. This research begins to unravel the FMRP dysregulation within TSC-deficient neurons and underlying mechanism of ASD in these diseases.

Disclosures: K.D. Winden: None. **T. Pham:** None. **M. Sahin:** 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.; Novartis, Biogen, Astellas, Aeovian, Bridgebio, and Aucta. Other; Novartis, Roche, Regenxbio, SpringWorks Therapeutics, Jaguar Therapeutics and Alkermes.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.05

Topic: A.07. Developmental Disorders

Support: NINDS R01 N2082635
NINDS R01 NS121718
NIH RISE Grant 5 R25 GM 59994-19

Title: Ubiquilin-1 Rescued Mutant $GABA_A$ Receptor Subunit Mediated Developmental Epileptic Encephalopathies

Authors: *G. I. NWOSU[1], W. SHEN[2], J. KANG[2];
[1]Meharry Med. Col., Nashville, TN; [2]Neurol., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: The β_3 subunit of the $GABA_A$ receptor is abundantly expressed during the development of the central nervous system. Mutations of this subunit have been linked to Lennox-Gastaut Syndrome (LGS) in humans. The impact of a mutation of this subunit and how it can contribute to a developmental and epileptic phenotype are poorly understood, let alone mechanism-based treatment. Ubiquinlin-1(Plic-1) is an adaptor protein between ubiquitin and the proteasome, that has been reported to stabilize the β_3 subunit. Preliminary work in the lab has shown that the overexpression of Plic-1 can rescue mutant subunit containing receptors. We have developed a novel mouse model of LGS (*$Gabrb3^{+/N328D}$*) to characterize the major defects caused by the mutation from molecular to neurobehavioral levels. We will determine if overexpression of Plic-1 can rescue the molecular and functional phenotypes of the mutant mice. Expression of the α_1, β_3, and γ_2 subunit configuration of the $GABA_A$ receptor in total lysates, cell surface level, and synaptosomes will be analyzed in mice with or without overexpression of Plic-1. Video monitoring and synchronized EEG recordings will be conducted to evaluate the effect of overexpression of Plic-1 on seizure activity with emphasis on spike wave discharge electroencephalogram patterns. The expression of β_3 subunits was reduced in both total lysates and synaptosomes in the *$Gabrb3^{+/N328D}$*. Plic-1 increased the expression of mutant β3 subunit containing receptors in vitro and in $Gabrb3^{+/N328D}$ mice. Plic-1 has also shown to reduce the seizure activity as reflected in the Plic-1 and $Gabrb3^{+/N328D}$ co-expressing mice.

Disclosures: G.I. Nwosu: None. **W. Shen:** None. **J. Kang:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.06

Topic: A.07. Developmental Disorders

Support: KGM5222113
NRF-2019M3C7A1031534 (D.Y.L.)
NRF-2015M3C7A1029113 (J.-R.L.)
NRF-2019R1I1A2A01063642 (D.Y.L.)

Title: Altered gene expression profiles in neural stem cells derived from Duchenne Muscular Dystrophy patients with intellectual disability

Authors: *J. KOO[1], S. PARK[2], S.-E. SUNG[5], J. LEE[6], D. KIM[3], J. LEE[4], J.-R. LEE[1], N.-S. KIM[1], D. LEE[1];
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Abstract: Intellectual disability (ID) is a neurodevelopmental disorder defined by below-average intelligence (intelligence quotient of <70) accompanied by adaptive behavior deficits. Defects in the functions of neural stem cells during brain development are closely linked to the pathogenesis of ID. To understand the molecular etiology of ID, we examined neural stem cells from individuals with Duchenne muscular dystrophy (DMD), a genetic disorder in which approximately one-third of the patients exhibit ID. In this study, we generated induced pluripotent stem cells from peripheral blood mononuclear cells from a normal individual and DMD patients with and without ID to identify ID-specific functional and molecular abnormalities. We found defects in neural ectoderm formation in the group of DMD patients with ID. Our transcriptome analysis of patient-derived neural stem cells revealed altered expression of genes related to the hippo signaling pathway and neuroactive ligand-receptor interaction, implicating these in the pathogenesis of ID in patients with DMD.

Disclosures: J. Koo: None. **S. Park:** None. **S. Sung:** None. **J. Lee:** None. **D. Kim:** None. **J. Lee:** None. **J. Lee:** None. **N. Kim:** None. **D. Lee:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.07

Topic: A.07. Developmental Disorders

Support: PI21/00488
RD21/0009/0008

Title: Cannabidiol-induced modulation of behavioral, cellular and gene expression changes in an animal model of FASD

Authors: *A. GASPARYAN[1], D. NAVARRO[2], F. NAVARRETE RUEDA[3], A. AUSTRICH-OLIVARES[4], G. ACOSTA[5], J. MANZANARES[6];
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Spain; [5]Inst. de Neurociencia Cognitiva y Traslacional (INCyT), Buenos Aires, Argentina; [6]Univ. Miguel Hernandez, San Juan de Alicante, Spain

Abstract: Fetal alcohol spectrum disorder (FASD) includes various neuropsychiatric disturbances related to gestational and lactational ethanol exposure. Available treatments are minimal and do not modulate ethanol-induced damage. Developing animal models simulating FASD could be essential for understanding the underlying brain alterations and searching for efficient therapeutic approaches. Thus, the main goal of this study was 1) to develop a new animal model of FASD with associated long-lasting emotional, cognitive, cellular and molecular changes in males and females exposed to ethanol during gestation and lactation, and 2) to analyze early and chronic CBD administration effects on these ethanol-induced disturbances in males and females. For this purpose, C57BL/6J female mice were exposed to an ethanol voluntary consumption paradigm (28 days), selecting only those with higher ethanol consumption and preference to cross them with males. After gestation confirmation, oral ethanol gavage administration at a dose of 3 g/kg/12h (p.o.) started at gestational day 7 until the pup's weaning at postnatal day 21. On the weaning day, pups were separated by sex and CBD administration began (30 mg/kg/day, i.p.). After 4-6 weeks of treatment, behavioral, relative gene expression and immunohistochemical protein changes were analyzed. Rodents exposed to the animal model of FASD showed higher anxiety and depressive-like behaviors in the light-dark box, novelty-suppressed feeding and tail suspension tests, and a higher emotional reactivity in the acoustic startle response evaluation. In addition, cognitive impairment was observed in the novel object recognition and step-down inhibitory avoidance tests. These behaviors were accompanied by alterations on the stress axis and cannabinoid receptor gene expressions. In addition, an essential reduction of different neuronal markers in the hippocampus was observed by immunohistochemical analyzes. Interestingly, CBD not only normalized FASD model-induced emotional and cognitive disturbances but also gene expression changes with sex-dependent differences. The administration of CBD normalized the number of neurons, BDNF-positive cells, neurofilaments and glutamatergic terminals in the hippocampus. These results suggest that the repeated administration of CBD modulated the long-lasting behavioral and the gene and protein alterations induced by the FASD model. These results stimulate the possibility to perform clinical trials to evaluate the effects of CBD in children affected with Alcohol spectrum disorders.

Disclosures: A. Gasparyan: None. **D. Navarro:** None. **F. Navarrete Rueda:** None. **A. Austrich-Olivares:** None. **G. Acosta:** None. **J. Manzanares:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.08

Topic: A.07. Developmental Disorders

Support: Ministero della salute, Ricerca Corrente 2022
Fondazione Just Italia
Associazione Smith-Magenis Italia

Title: Retinoic acid-induced 1 gene haploinsufficiency deregulates lipid metabolism and causes autophagy defects in Smith-Magenis Syndrome

Authors: *J. ROSATI[1], E. TURCO[1], A. GIOVENALE[1], L. SIRENO[3], L. GORACCI[4], A. DI VEROLI[4], D. D'ANDREA[5], L. BERNARDINI[1], C. MARCHIORETTI[6], M. DELLA MONICA[7], A. NARDONE[8], G. ZAMPINOI[9], R. ONESIMO[10], F. CAICCI[11], D. FERRARI[12], M. PENNUTO[6], A. VESCOVI[2];
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Abstract: Background: Smith-Magenis syndrome (SMS) is a neurodevelopmental disorder characterized by cognitive and behavioral symptoms, obesity, and sleep disorder. There is no therapy to alleviate symptoms or delay disease onset. SMS is due to the haploinsufficiency of the retinoic-acid-induced-1 gene (RAI1) caused by either chromosomal deletions (SMS-del) or RAI1 missense/nonsense mutations. Little is known about the molecular mechanisms underlying this disease.**Aim of the study:** The purpose of this work was to identify the key molecular mechanisms involved in SMS pathogenesis and/or progression aspects that characterize the syndrome. We compared the phenotype of SMS fibroblasts derived from SMS patients with those obtained with fibroblasts derived from healthy subjects, in order to uncover SMS-intrinsic alterations of cell functionality. Subsequently, we corrected the aberrant phenotype with molecules acting on the identified deregulated pathways. **Materials and methods:** We generated and characterized primary cells derived from skin biopsies of four SMS patients (two carrying the SMS-del, two with RAI1 point mutations), and four healthy controls. We used omic analyses to characterize transcriptional and lipidomic profiles and immunofluorescent analyses and western blotting to evaluate cellular behavior. Results: We found an altered expression of genes involved in lipid synthesis/catabolism and lysosomal function associated with a deregulation of lipid metabolism, accumulation of lipid droplets, and a block of autophagy flux, in SMS fibroblasts compared to controls. Furthermore, SMS cells showed increased cell death associated with mitochondrial dysfunction and reactive oxygen species production. Treatment with N-acetyl-cysteine reduced the rate of cell death and lipid accumulation but it did not improve the autophagy flux.**Conclusion:** Here, we demonstrated for the first time, in a human model of Smith-Magenis Syndrome, that this disease is characterized by pathological pathways such as lipid dysmetabolism, autophagy defects and mitochondrial dysfunction, all representing new potential therapeutic targets for patient treatment.

Disclosures: J. Rosati: None. **E. Turco:** None. **A. Giovenale:** None. **L. Sireno:** None. **L. Goracci:** None. **A. Di Veroli:** None. **D. D'Andrea:** None. **L. Bernardini:** None. **C. Marchioretti:** None. **M. Della Monica:** None. **A. Nardone:** None. **G. Zampinoi:** None. **R. Onesimo:** None. **F. Caicci:** None. **D. Ferrari:** None. **M. Pennuto:** None. **A. Vescovi:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.09

Topic: A.07. Developmental Disorders

Support: NHMRC #1165616
Homer Hack Foundation

Title: Investigation of a novel de novo human homer1 mutation confirms the requirement of the c-terminal coiled-coil for homer1 function in developing neurons

Authors: *D. BLIGH, L. FOA, R. GASPERINI;
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Abstract: The Homer proteins play critical roles in regulating intracellular signalling. Certain Homer1 (H1) isoforms possess a coiled-coiled (CC) domain which allow Homer proteins to align in parallel tetramers, but the full extent of their importance for H1 function is unclear. We sought to investigate whether a novel de novo human H1 CC mutation (R297W) impairs tetramer formation and what its consequences were for H1 function. We subjected sensory neurons from E18.5 rat dorsal root ganglia to an in vitro growth cone turning assay against a Ca^{2+}-dependent guidance cue, brain derived neurotrophic factor (BDNF). We found that expression of R297W, but not WT H1, converts attractive growth cone turns towards a BDNF gradient to a repulsive response (one-way ANOVA with multiple comparisons p=<0.0001), suggesting that the H1 dependency for Ca^{2+}-dependent guidance previously reported relies upon a functional CC. Because BDNF guidance requires store operated calcium entry (SOCE), we recorded SOCE in E18.5 rat sensory neuron growth cones and hippocampal neurons using ratio metric Ca^{2+} imaging. We found that R297W expression significantly blunts SOCE amplitude in R297W expressing cells as compared to WT H1 expressing and untransfected controls (one-way ANOVA with multiple comparisons p=0.0061). R297W expression had similar effects upon SOCE amplitude to that of knockdown of endogenous H1 with siRNA, suggesting that the CC may render the mutant isoform non-functional. We hypothesised that the CC mutation impairs its ability to position endoplasmic reticulum-Ca^{2+} channel proteins like 1,4,5-trisphosphate receptors (IP_3R) near to SOC proteins such as stromal interaction molecules 1/2 (STIM1/2) and mGluR5 and link their function. Using direct stochastic optimal reconstruction microscopy (dSTORM), we investigated whether the correlation of R297W with these ligands differed from WT H1. With statistical object distance analysis, we investigated whether distances between R297W Homer, IP_3R, mGluR5 and STIM1/2 differed from those of WT H1 to determine whether R297W exists in shorter monomer or dimer forms and fails to form tetramers. We re-examined whether the Ca^{2+}-signalling defects and phenomena observed with dSTORM were present in induced pluripotent stem cells (IPSCs) differentiated into neurons and derived from the carrier of R297W. Finally, we tested whether the R297W mutation was responsible for the changes observed, by CRISPR repairing the mutation in IPSCs. Our findings shed new light

upon the importance of the CC domain for H1 function and demonstrate for the first-time biochemical alterations caused by a de novo human Homer mutation.

Disclosures: D. Bligh: None. **L. Foa:** None. **R. Gasperini:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 606.10

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS121130

Title: Genetic loss of the Sez6 family results in motor and cognitive impairments that are not complement dependent

Authors: N. SILVER, A. HOBBINS, J. RANDOLPH, A. STOUT, ***J. HAMMOND**; Univ. of Rochester, Rochester, NY

Abstract: The Sez6 gene family consists of Sez6, Sez6L, and Sez6L2. They are all highly expressed by neurons and have each been identified as potential susceptibility genes for multiple neurodevelopmental and psychiatric disorders. Sez6 family triple knockout (TKO) mice have impaired cognition and motor functions. As Sez6 proteins were recently found to be complement inhibitors, some of the altered phenotypes of Sez6 TKO mice could be downstream of excessive complement activation and elevated complement-mediated synaptic pruning. We tested whether the deficits of Sez6 TKO mice are complement-dependent by comparing them to Sez6 TKO mice crossed with complement component C3 knockout (KO) mice. Sez6 TKO mice were significantly impaired compared to WT mice and C3 KO mice in their rotarod performance, gait parameters, nesting, marble burying, social preference, acoustic startle response, and fear conditioning. However, Sez6 TKO/C3 KO showed no improvements in the battery of behavioral tests compared to Sez6 TKOs. In some behavioral tests, the Sez6 TKO/C3 KOs were even more impaired. These data suggest that Sez6 family proteins have important molecular functions independent of complement inhibition and that their interaction with complement may be more complicated than the simple inhibitor relationship suggested by *in vitro* studies.

Disclosures: N. Silver: None. **A. Hobbins:** None. **J. Randolph:** None. **A. Stout:** None. **J. Hammond:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Program #/Poster #: 606.11

Topic: A.07. Developmental Disorders

Support: Jordan's Guardian Angels
State of California 2018-2019 Budget SB 840
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NIMH T32 in Learning Memory and Plasticity 5T32MH112507-04

Title: Crispr/dcas13b site-directed rna editing of pathogenic adenosine nucleotides implicated in genetically-linked intellectual disabilities

Authors: *J. L. CARTER[1], J. A. HALMAI[1], J. WALDO[1], J. NOLTA[2], K. FINK[1];
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Abstract: Pathogenic G-to-A mutations that manifest as neurological disorders have traditionally been challenging to edit in neuronal cells as current strategies require host-cell DNA repair pathways and incorporation of a template sequence. ADAR2 is a brain-enriched protein which post-transcriptionally modifies double-stranded RNA (dsRNA) by deaminating adenosine nucleotides to inosine, thereby enabling translational machinery to create a functional adenosine to guanosine edit within the transcript. The CRISPR associated protein 13 (dCas13b) is a catalytically inactive RNA binding protein which can achieve site-directed RNA editing when fused to the ADAR2 deaminase domain (ADAR2DD). A guide RNA (gRNA) sequence forms a dsRNA substrate for dCas13b-ADARDD2 binding at the complementary target while enabling RNA editing at a single base. However, the CRISPR Cas13 system has yet to be employed for RNA editing of G-to-A mutations in neuronal cells. Our lab created induced pluripotent stem cell (iPSC) from fibroblasts of individuals with a pathogenic G-to-A mutation in exon 5 (E198K) of protein phosphatase 2, regulatory subunit B', delta (*PPP2R5D)* which are causative for the neurodevelopmental disorder Jordan's Syndrome. Here, we have differentiated isogenic and variant iPSCs to neural stem cells (NSCs) to evaluate RNA editing efficiency and efficacy. Towards this goal, we have designed and screened exon 5 (E198K) gRNA to identify lead gRNA which have high on-target editing and low-to-moderate off-target editing in the *PPP2R5D* transcript. We demonstrate gRNA length as an important factor in on-target editing efficiency. We have differentiated isogenic and E198K variant NSCs to a mixed neuronal population or midbrain neurons to understand the consequence of G-to-A mutations on *PPP2R5D* targets at the phosphoproteomic and transcriptomic levels. In addition, we assess iPSC-derived neuronal activity at a population level with multielectrode array recordings (MEA). Our future work is focused on employing the CRISPR RNA editor system in these patient-specific neuronal models. These studies support the potential for the CRISPR/dCas13b system to selectively edit mutant transcripts harboring G-to-A mutations in neuronal cells while providing an alternative editing technology for neurodevelopmental disorders.

Disclosures: J.L. Carter: None. **J.A. Halmai:** None. **J. Waldo:** None. **J. Nolta:** None. **K. Fink:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Program #/Poster #: 606.12

Topic: A.07. Developmental Disorders

Support: NIH grant DP2 MH122398

Title: Mosaic Cas9 fusions to investigate cortical wiring by IgLON schizophrenia risk-genes in the rodent brain

Authors: *A. ROMANOWSKI[1], B. ALTAS[1], R. R. RICHARDSON[1], S. N. KHIM[1], E. A. CARAKER[2], A. POULOPOULOS[1];
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Abstract: Large-scale patient sequencing suggests that neuropsychiatric genetics often involve many gene variants affecting subtle regulation in expression of risk genes. These affect the timing and dosage of gene expression rather than the protein structure of gene products. In order to study the consequences of polygenic schizophrenia risk gene dysregulation in the rodent brain, we developed parallel approaches using in utero electroporation of Cas9 fusions to investigate which risk genes are involved in which aspects of cortical wiring, and how these are impacted by schizophrenia-related dysregulation. Using a CRISPR knockout approach, we identify risk genes NEGR1, LSAMP, and Neurotrimin of the IgLON cell adhesion molecule family to be implicated in intracortical circuit wiring. Using a Cas9 fusion knockin approach, we investigate the subcellular localization and in vivo local interactome of IgLON proteins. Finally we develop Cas9 fusion agents to manipulate the expression of IgLON genes in vivo to model gene dosage effects within the range seen in patients with schizophrenia. We demonstrate that these three in utero CRISPR/Cas9 fusion approaches together establish a generalizable platform to investigate the complex genomic underpinnings of typical and atypical brain wiring across neurodevelopmental and neuropsychiatric conditions.

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Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.13

Topic: A.07. Developmental Disorders

Support: NIMH R01 084989

Title: Fmrp coordinates bmp and insulin signaling for glial clearance of developmentally-transient brain neurons

Authors: *C. SONG[1], K. BROADIE[1,2,3,4];
[1]Biol. Sci., Vanderbilt Univ., Nashville, TN; [2]Kennedy Ctr. for Res. on Human Develop., [3]Dept. of Cell and Developmental Biol., Vanderbilt Med. Ctr., Nashville, TN; [4]Vanderbilt Brain Inst., Nashville, TN

Abstract: Fragile X syndrome (FXS) is a leading neurodevelopmental disorder disease caused by loss of Fragile X Mental Retardation Protein (FMRP). In the *Drosophila* FXS model, neuronal FMRP is required to activate glial phagocytosis for the clearance of developmentally-transient neurons. Here, we discover that neuronal FMRP limits insulin receptor (InR) expression by restricting Bone Morphogenetic Protein (BMP) signaling which, in turn, regulates the neuronally-secreted "eat me" signal pretaporter (Prtp) and "digestion" signal β amyloid protein precursor-like (APPL) for glial phagocytosis. Previous published work indicates secreted, cleaved APPL ingested by glia activates glial endosomal function. With RNA-immunoprecipitation, qPCR and Western blot assays, we find that loss of FMRP elevates transcription factor mothers against decapentaplegic (Mad) mRNA levels to increase phosphorylated Mad (pMad) signaling. With ChIP assays, pMad binds protein kinase B (Akt) and InR. We find targeted reduction of pMad in neurons elevates phospho-Akt levels and reduces InR expression, phenocopying the loss of neuronal clearance caused by neuronal FMRP knockdown. Consistently, both neuronal FMRP and Mad knockdown cause decreased Prtp expression, an increased number of neuron-associated glia, and loss of neuronal clearance. As expected, neuronal knockdown of Akt and InR causes the opposite phenotypes. Based on these findings, we suggest FMRP works in the BMP and insulin signaling pathways to coordinately regulate neuron-to-glia "eat me" and "digestion" signals controlling glial phagocytosis during neuronal clearance. Taken together, we propose a FMRP-pMad-InR-APPL signaling cascade involved in glial-mediated clearance of developmentally-transient neurons in the *Drosophila* FXS disease model, suggesting potential new mechanisms and molecular targets for devising novel FXS patient treatments.

Disclosures: C. Song: None. **K. Broadie:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: T32 5T32AG057468
Wolverine Foundation Grant

Dr. Ralph and Marian Falk Medical Research Trust
T32 GM079086

Title: Loss of MAPK8IP3 affects endocytosis in Neurons

Authors: *A. M. SNEAD[1], S. GOWRISHANKAR[2];
[1]Univ. of Illinois at Chicago, [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Perturbations in endo-lysosomal trafficking pathways are linked to many neurodevelopmental and neurodegenerative diseases. Of relevance to our current study, MAPK8IP3/JIP3, a brain enriched putative adaptor between lysosomes and motors has been previously implicated as a key regulator of axonal lysosome transport. Since de novo variants in MAPK8IP3 have recently been linked to a neurodevelopmental disorder with intellectual disability, there is a need to better understand the functioning of this protein in human neurons. To this end, using induced neurons (i3Neurons) derived from human iPSCs lacking MAPK8IP3, we demonstrate that loss of hMAPK8IP3 affects endocytic uptake in neurons but does not affect the proteolytic activity of lysosomes in neuronal cell bodies. Our findings indicate that MAPK8IP3 may be a regulator of bulk endocytosis in neurons and that altered endocytic uptake may play a role in MAPK8IP3-linked neurodevelopmental disorders.

Disclosures: A.M. Snead: None. **S. Gowrishankar:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.15

Topic: A.07. Developmental Disorders

Title: Functional dissection of developmental regulators in the human neuroectoderm

Authors: *A. AMIRI[1], D. BAKER[1,4], P. KUMAR[2], W. F. FLYNN[1], J. MCDONOUGH[3], W. C. SKARNES[3,5], P. ROBSON[1,5];
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Abstract: The emergence of human iPSC-derived models of human brain development not only help overcome the challenges in accessing human fetal tissues but provides a dynamic cellular system to functionally investigate the role of developmental regulators. Functionally charactering such transcriptional regulators and where they bind in the genome is directly relevant to understanding the genetics of neurodevelopmental disorders. We are establishing a molecular and cellular phenotyping pipeline for the analysis of null alleles, generated in KOLF2.1 (46;XY) hiPSCs, of neurodevelopmental regulators. Utilizing our established forebrain-directed hiPSC differentiation scheme, we have generated a time-course series of single cell RNA-seq (scRNAseq) to capture the transcriptional dynamics of neuroectoderm formation. Suspension

cultures transition through epiblast states (Day 0, 1 and 2) to mostly forebrain fate at Day 4 and 6. At Day 4, some cells are representative of gastrulation-like states as evidenced by the presence of the rostral neuroectoderm and, on a separate trajectory, a small number of cells representing the primitive streak, axial mesoderm/notochord and endoderm. At Day 6, expression of *PAX6* and numerous other neural transcription factors were evident. Notably, a small population of post-mitotic excitatory deep cortical layer neurons was evident at this stage. Utilizing *PAX6* as a proof-of-concept, we first experimented with null allele generation strategies, comparing a premature termination codon +1 base frame shift (PTC+1) strategy with a comprehensive coding sequence deletion (KO) strategy. Both led to undetectable PAX6 protein in brain organoids. Bulk RNA-seq at Day 8 and 9 of wt, PTC+1, and KO lines indicated significant gene expression differences (min. 317 genes at $p<0.05$) between the null and wt lines, inclusive of known and novel PAX6 target genes. While there was a complete absence of reads mapping to the KO region, 5' and 3' reads of the *PAX6* transcript were detectable, and significantly higher in expression compared to wt, suggesting repressor elements were lost upon removal of the KO region. Reads mapping across the entire *PAX6* transcript were detectable in the PTC+1 clones but significantly lower than in wt, likely a result of nonsense-mediated decay (NMD) expected to occur with this strategy. While PAX6 null clones, in comparison to wt, were in a less differentiated state, we also detected a shift in forebrain patterning with fewer telencephalic and more diencephalic markers in PAX6 null clones. Currently we are generating a time-series of scRNAseq and single nucleus ATAC-seq to better resolve these altered cellular states.

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Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Program #/Poster #: 606.16

Topic: A.07. Developmental Disorders

Support: 5R01MH102603-04, PA18-586
1R01MH126481
F31MH123140
HHMI Gilliam

Title: Cell-type-specific regulation of neuronal progenitor cell development by Foxp1 in the developing murine neocortex

Authors: *A. ORTIZ, F. AYHAN, M. HARPER, G. KONOPKA;
Neurosci., UT Southwestern, Dallas, TX

Abstract: Cortical development is a tightly controlled process and any deviation during its unfolding may increase the susceptibility to neurodevelopmental disorders, such as autism

spectrum disorders (ASD). Numerous studies have identified mutations in FOXP1, a gene encoding a transcription factor enriched in the developing and mature neocortex, as causal for ASD. Our group has previously shown that loss of *Foxp1* in the mouse neocortex leads to reduced total cortical thickness, alterations in cortical lamination, and changes in relative thickness of cortical layers. However, the mechanisms underlying these changes remain unclear. My work characterizes the developmental requirement of neocortical *Foxp1* throughout early development using a cortical-specific conditional knock-out of *Foxp1* (*Foxp1* cKO) in mice. Foxp1 expression is temporally regulated in distinct cell types throughout development. We measure cell-type specific transcriptomic changes in the cortex of *Foxp1* cKO mice during early development and report that early loss of *Foxp1* results in alterations of the developmental trajectory of neuronal progenitor cells when compared to controls. These changes are cell-type-specific and provide insight into how Foxp1 regulates specific cell types to impact subsequent neuronal generation from an early stage of cortical development. This aspect may contribute to the post-natal differences seen in cortical layers and relative cortex thickness. This adds to other ongoing work of genomic profiling, of identifying potential key driver genes, assessing how loss of *Foxp1* alters the proliferative capacity of radial glia cells and cell migration, and cell autonomous and non-autonomous effects.

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Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Program #/Poster #: 606.17

Topic: A.07. Developmental Disorders

Support: Harvard Brain Science Initiative Bipolar Disorder Seed Award
NIH Grant RO1 MH101148

Title: Regulation of the Wnt signaling pathway by the neural-specific transcription factor POU3F2

Authors: *C. R. BENOIT[1], M.-C. LIAO[2], R. V. PEARSE[2], A. HE[2], S. FANCHER[2], T. L. YOUNG-PEARSE[2];
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Abstract: Proper neurodevelopment requires an intricate series of gene regulatory interactions, mediated by the activity of key neural transcription factors. POU3F2, a neural-specific POU-domain transcription factor, is critical for the proper distribution of specific neuronal fates, including layer II-III cortical neurons and hypothalamic neurons of the supraoptic and paraventricular nuclei. In humans, heterozygous loss-of-function and missense mutations in *POU3F2* have been linked to neurodevelopmental defects. Additionally, *POU3F2* has been identified as a candidate risk gene for neuropsychiatric disorders such as bipolar disorder, autism

spectrum disorder, and schizophrenia. While these studies have underscored the importance of proper POU3F2 activity during neurodevelopment, little is known about its molecular function or downstream effectors. To elucidate the POU3F2-dependent regulatory network, we generated loss-of-function mutations in *POU3F2* in human induced pluripotent stem cell (iPSC) lines, which were differentiated into neural progenitor cells (NPCs). RNA-sequencing of $POU3F2^{WT}$ and $POU3F2^{MUT}$ NPCs identified downregulation of Wnt pathway inhibitors as a central hallmark in $POU3F2^{MUT}$ NPCs, validated by a Wnt reporter assay demonstrating increased canonical Wnt responsiveness in $POU3F2^{MUT}$ NPCs that is independent of β-catenin stabilization. These data were integrated with correlation analyses in a large cohort of human iPSC-derived NPCs exhibiting natural variation in POU3F2 levels, which led to the identification of three known inhibitors of Wnt signaling as potential downstream effectors of POU3F2 (AXIN2, SOX13, ZNRF3). Single-cell RNA-sequencing demonstrated that $POU3F2^{MUT}$ NPCs exhibit a shift in NPC subtype favoring increased levels of early, ventricular zone-enriched progenitors, which is accompanied by a corresponding increase in canonical Wnt signaling. From these data, we hypothesize that POU3F2 activates transcription of canonical Wnt pathway inhibitors to regulate the Wnt signaling pathway, and that loss of POU3F2 results in enhanced Wnt activation and a corresponding shift in NPC subtype. We plan to further examine POU3F2-mediated regulation of the Wnt signaling pathway and its role in the etiology of neurodevelopmental disorders in future work, using both iPSC-derived cellular models and mouse models.

Disclosures: C.R. Benoit: None. **M. Liao:** None. **R.V. Pearse:** None. **A. He:** None. **S. Fancher:** None. **T.L. Young-Pearse:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.18

Topic: A.07. Developmental Disorders

Title: Hook3 interacts with mTOR to regulate neuronal development

Authors: *N. GHANI, T. FAMILARA, E. DAHLMANN, A. TANG, C. CHANG, E. OH, V. VO;
Nevada Inst. of Personalized Med., Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Hook microtubule tethering protein 3 (Hook3) is a Golgi-localized protein that functions in cellular trafficking and the binding of microtubules to organelles. Hook3 is associated with poor prostate cancer prognosis and has a role in regulating amyloid beta production in Alzheimer's disease. Hook3 is expressed in neurons and regulates the timing of neurogenesis. Additionally, Hook3 aggravates hypoxia-reoxygenation induced inhibition of the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway in H9c2 rat cardiomyoblasts, and possibly neural stem cells. Given a

genetic interaction between Hook3 and the mTOR signaling pathway, we hypothesize that this unique module is required to regulate neural stem cell development. Through mass spectrometry analysis, we identified proteins in the mTOR pathway as interactors of Hook3. Here, we show that knockdown of Hook3 in neural progenitor cells affects cell migration and protein trafficking. We will use immunochemistry techniques to characterize the interaction of Hook3 with proteins in the mTOR pathway and demonstrate a role for Hook3 in the regulation of neural cells.

Disclosures: **N. Ghani:** None. **T. Familara:** None. **E. Dahlmann:** None. **A. Tang:** None. **C. Chang:** None. **E. Oh:** None. **V. Vo:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.19

Topic: A.07. Developmental Disorders

Title: Regulation of the excitatory synapse through USP7

Authors: *H. BAKER[1], J. ITORRALBA[1], S. BOSS[1], J. ROSALES OLAYA[1], C. CHANG[2], V. VO[2], E. OH[1];
[2]Nevada Inst. of Personalized Med., [1]Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Loss of function lesions in Ubiquitin-specific protease (USP) 7 are associated with intellectual disability, speech delay, autism spectrum disorder, and seizures. While the deubiquitinating activity of USP7 has been characterized in cancer, its role in neurological conditions is not understood. Here, we show that USP7 can regulate the development of the brain by localizing to synaptosomes in the hippocampus and stabilizing neural-specific substrates. Using mass spectrometry, we identify discrete protein complexes that interact with USP7 and are necessary for functions such as signaling, vesicular trafficking, Golgi assembly, and cell polarity at the synapse. Using immunoprecipitation, we show that some missense mutations in USP7 abolish these interactions. Alternating USP7 levels in rat neurons through chemical inhibition and overexpression results in the perturbation of neural synchrony and network oscillations. Taken together, our data suggest that USP7 regulates synaptic function during development.

Disclosures: **H. Baker:** None. **J. Itorralba:** None. **S. Boss:** None. **J. Rosales Olaya:** None. **C. Chang:** None. **V. Vo:** None. **E. Oh:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: Department of Defense (Project Title: A Subependymal Giant Cell Astrocytoma (SEGA) Mouse Model Sponsor Name: US Army Medical Research Sponsor Award Number: W81XWH2010447

Title: Tsc2 shapes olfactory bulb granule cell morphological and molecular characteristics

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

Abstract: Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations that inactivate *TSC1* or *TSC2*. Hamartin and tuberin are encoded by *TSC1* and *TSC2* which form a GTPase activating protein heteromer that inhibits the Rheb GTPase from activating a growth promoting protein kinase called mammalian target of rapamycin (mTOR). Growths and lesions occur in the ventricular-subventricular zone (V-SVZ), cortex, olfactory tract, and olfactory bulbs (OB) in TSC. A leading hypothesis is that mutations in inhibitory neural progenitor cells cause brain growths in TSC. OB granule cells (GCs) are GABAergic inhibitory neurons that are generated through infancy by inhibitory progenitor cells along the V-SVZ. Removal of *Tsc1* from mouse OB GCs creates cellular phenotypes seen in TSC lesions. However, the role of *Tsc2* in OB GC maturation requires clarification. Here, it is demonstrated that conditional loss of *Tsc2* alters GC development. A mosaic model of TSC was created by performing neonatal CRE recombinase electroporation into inhibitory V-SVZ progenitors which yielded clusters of ectopic cytomegalic neurons with hyperactive mTORC1 in homozygous *Tsc2* mutant but not heterozygous or wild type mice. Similarly, homozygous *Tsc2* mutant GC morphology was altered at postnatal days 30 and 60. *Tsc2* mutant GCs had hypertrophic dendritic arbors that were established by postnatal day 30. In contrast, loss of *Tsc2* from mature GCs had no to only modest effects on mTORC1, soma size, and dendrite arborization. OB transcriptome profiling revealed a network of significantly differentially expressed genes following loss of *Tsc2* during development that altered neural circuitry. These results demonstrate that *Tsc2* has a critical role in regulating neural development and shapes inhibitory GC morphological and molecular characteristics.

Disclosures: J.C. Holmberg: None. **V.A. Riley:** None. **A.M. Sokolov:** None. **D.M. Feliciano:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.21

Topic: A.07. Developmental Disorders

Support: NIH Grant 1F31EY031577-01
NIH Grant 1F99NS125819-01

Title: The role of Vsx2 in human retinal organoid development

Authors: *V. HONNELL, S. SWEENEY, M. DYER;
St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Super-enhancers (SEs) are expansive regions of genomic DNA comprised of multiple putative enhancers that contribute to dynamic gene expression patterns during development. This is particularly important in neurogenesis because many essential transcription factors have complex developmental stage- and cell-type specific expression patterns across the central nervous system. In the developing retina, *Vsx2* is expressed in all retinal progenitor cells and is maintained in differentiated bipolar neurons and Müller glia. Mutations in the *Vsx2* gene cause microphthalmia in humans and mice because it is required for retinal progenitor cell proliferation. Due to this severe early developmental phenotype, it has been difficult to elucidate the role of Vsx2 in bipolar neuron and Müller glia differentiation. Through scATAC-seq., scRNAseq., and immunohistochemistry, we found that a single SE controls this complex and dynamic pattern of expression in mice. The deletion of one region disrupts retinal progenitor cell proliferation in early retinal development. The deletion of another region has no effect on retinal progenitor cell proliferation but instead leads to a complete loss of bipolar neurons. We hypothesize that this pattern of expression will be recapitulated in a model of human retinal development. To test this hypothesis, we generated retinal organoids from human embryonic stem cells (hESCs) with the region deletions previously examined in mice. We analyzed bulk gene expression, cell type presence, and retinal progenitor cell proliferation using RNA-sequencing, immunofluorescence, and EdU labeling across multiple stages of retinal organoid development. These datasets will offer insights into the enhancer regions regulating *Vsx2* gene expression in humans and could identify previously unknown enhancer candidates contributing to microphthalmia.

Disclosures: V. Honnell: None. **S. Sweeney:** None. **M. Dyer:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Program #/Poster #: 606.22

Topic: A.07. Developmental Disorders

Support: NIH/NIMH R01MH102603
Simons Foundation for Autism Research 573689
T32-HL139438 NHLBI Institutional Training Grant
NIH/NIMH 1F31NS117030-01

Title: Genetic robustness of FOXP transcription factors help maintain proper striatal function

Authors: *N. I. AHMED, N. KHANDELWAL, A. KULKARNI, K. SIVAPRAKASAM, A. ANDERSON, J. GIBSON, G. KONOPKA;
Univ. of Texas Southwestern Med. Ctr., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Variants in the transcription factors *FOXP1* and *FOXP2* are significantly associated with autism spectrum disorder (ASD) and expressive language impairments. Both genes are expressed in spiny projection neurons (SPNs) in the striatum, where they may work together to regulate gene expression. Knockout of *Foxp1* from the dopamine 2 receptor (D2) SPNs in mice results in significant behavioral, morphological, and physiological impairments, while there are fewer changes in all of these domains upon deletion of *Foxp1* from the dopamine 1 receptor (D1) SPNs. This difference may be due to the differential expression of *Foxp1* and *Foxp2* in the SPNs; *Foxp1* is highly expressed in both D1 and D2 SPNs whereas *Foxp2* is more highly expressed in the D1 SPNs relative to D2 SPNs. Therefore, we hypothesize that *Foxp1* and *Foxp2* have compensatory functions in the D1 SPNs. Utilizing mice that have a Drd1 specific knockout of *Foxp1*, *Foxp2*, or both genes, we find that loss of both genes results in impaired motor learning, hypoactivity, and social behavior as well as increased firing of the D1 SPNs. Differential gene expression analysis from single nuclei RNA-sequencing implicates genes involved in ASD risk, maintenance of electrophysiological properties, and neuronal development and function. These data support the hypothesis that Foxp1 and Foxp2 functionally compensate for each other in D1 striatal neurons.

Disclosures: N.I. Ahmed: None. **N. Khandelwal:** None. **A. Kulkarni:** None. **K. Sivaprakasam:** None. **A. Anderson:** None. **J. Gibson:** None. **G. Konopka:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.23

Topic: A.07. Developmental Disorders

Support: NRF-2020M3E5D9079908
NRF-2020R1A2C2005021

Title: Vps13b regulates morphology of mitochondria and clearance of damaged mitochondria

Authors: *S.-K. LEE[1], J.-A. LEE[1], J.-Y. MOON[2], D.-J. JANG[3], H.-J. HAM[1], H. CHOI[1], S. PARK[1], S.-W. PARK[3];
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Abstract: <META NAME="author" CONTENT="이수경">
VPS13B regulates morphology of mitochondria and mitophagy<u>**Soo-Kyeong Lee[1]**</u>**, Hyun-Ji**

Ham[1], Haneul Choi[1], Semin Park[1],Sang-Won Park[2], Ji-Young Moon[3], Deok-Jin Jang*[2], Jin-A Lee*[1][1] Department of Biological Sciences and Biotechnology, College of Life Sciences and Nanotechnology, Hannam University, [2]Department of Applied Biology, College of Ecology and Environment, Kyungpook National University[3] Neural circuit research group, Korea Brain Research Institute, Daegu, Korea

VPS13B mutations are associated with Cohen syndrome (CS) which is a rare autosomal recessive disease, also known as one of the genetic developmental disorders. VPS13B is a transmembrane protein and Golgi apparatus protein involved in vesicular trafficking. However, the cellular functions of VPS13B associated with cellular organelle is largely unknown. In this study, we found dysregulation of mitochondria Quality Control in VPS13B KO Hela cell line generated using CRISPR/Cas9 technology. Very interestingly, our electron microscopic analysis and immunostaining analysis showed that VPS13B KO cells have mitochondria with abnormal morphology compared to WT. Furthermore, membrane potential or dynamics of mitochondria was significantly impaired in VPS13B KO HeLa cell compared to WT indicating that VPS13B has a important roles on the regulation of mitochondria quality control. Since a quality control of abnormal mitochondria is associated with mitophagy, we further examined mitophagy in WT and VPS13B KO cells. Surprisingly, autophagic flux of GABARAPL1 was dramatically impaired by deficiency of VPS13B raising its possible roles on the regulation of basal mitophagy, leading to accumulation of mitochondrial proteins such as TIM23. Furthermore, we found that accumulation of Ubiquitinated parkin and PINK1 in VPS13B KO Hela cells treated with CCCP, and protein level of TAX1BP1 as a mitophagy receptor was significantly reduced. We are now investigating how VPS13B regulates basal mitophagy and parkin-mediated mitophagy.

(1) *Mol Brain* ,**2020**, doi.org/10.1186/s13041-020-00611-7, (2) *J. Clin. Med*. **2020**, doi.org/10.3390/jcm9061886, (3) *Cell death & disease*, **2020**, doi.org/10.1038/s41419-020-03165-7

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Disclosures: S. Lee: None. **J. Lee:** None. **J. Moon:** None. **D. Jang:** None. **H. Ham:** None. **H. Choi:** None. **S. Park:** None. **S. Park:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.24

Topic: A.07. Developmental Disorders

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UC CRCC seed grant C21CR2007
NSF CAREER award IOS-2143711
Canadian Institutes of Health Research FDN334023 and PJT471325

Brain Canada-Weston Foundation (to F.C. and M.C.)
Canada Foundation for Innovation 33768
National Science Foundation MRI Award DMR-1625733

Title: Cilium proteomics reveals Numb as a positive regulator of the Hedgehog signaling pathway

Authors: *X. LIU[1], P. T. YAM[2], W.-J. CHEN[2,3], S. SCHLIENGER[2,4], O. TORRES GUTIERREZ[1], E. CAI[1], J. ZHANG[1], A. Y. TING[5], T. C. BRANON[5], M. CAYOUETTE[2,4,6], F. CHARRON[2,4,6], X. GE[1];
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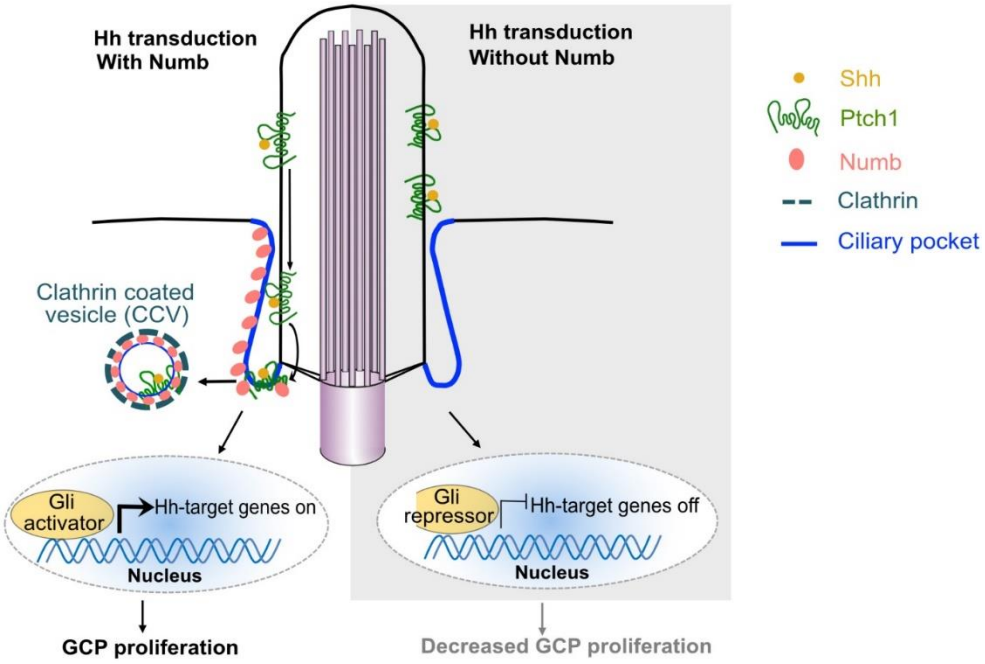
Abstract: The transduction of Hedgehog (Hh) signaling relies on the primary cilium, a cell surface organelle acting as a signaling hub for the cell. Using proximity labeling and mass spectrometry, we studied the ciliary proteome and identified Numb as a new ciliary protein that positively regulates the Hh pathway. Numb localizes to the ciliary pocket and acts as an endocytic adaptor to incorporate Ptch1 into clathrin-coated vesicles, thereby promoting Ptch1 exit from the cilium, a key step in Hh signaling activation. Numb loss impaired Sonic Hedgehog-induced Ptch1 departure from the cilium, resulting in severe attenuation of Hh signaling. Genetic ablation of *Numb* and its homolog *Numblike* in the developing cerebellum impaired the proliferation of granule cell precursors, a Hh-dependent process, resulting in reduced cerebellar size. This study demonstrates a key function of Numb in controlling protein levels in the cilium, and highlights Numb's critical role in the regulation of Hh signaling and Hh-dependent developmental events.



Disclosures: **X. Liu:** None. **P.T. Yam:** None. **W. Chen:** None. **S. Schlienger:** None. **O. Torres Gutierrez:** None. **E. Cai:** None. **J. Zhang:** None. **A.Y. Ting:** None. **T.C. Branon:** None. **M. Cayouette:** None. **F. Charron:** None. **X. Ge:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.25

Topic: A.07. Developmental Disorders

Support: Shriners Postdoctoral Fellowship 84303
NIH Grant R01NS113859
NIH Grant R01NS105886
NSF Grant 1754340

Title: Mechanisms of glutamate release during neural tube formation

Authors: R. GOYAL[1], *L. N. BORODINSKY[2];
[1]UC Davis, UC Davis, Sacramento, CA; [2]Univ. of California Davis, Univ. of California Davis, Sacramento, CA

Abstract: Failure of neural tube closure leads to one of the most common birth defects, known as neural tube defects (NTDs), which can have serious neurological consequences or be lethal. The use of antiepileptic drugs (AEDs), during pregnancy increases the incidence of NTDs. Our previous studies have shown that glutamate signaling through NMDA receptors is important for the formation of the neural tube and that the AED valproic acid perturbs this signaling, dysregulating neural plate cell proliferation and migration, and leading to NTDs.
The mechanism of glutamate release by neural plate cells is unclear since synapses are not assembled yet at these early stages of development. In this study we investigate the molecular mechanisms by which glutamate is released and signals in the folding neural plate of *Xenopus laevis* embryos.
To determine whether vesicular release of glutamate occurs in the neural plate we first assessed the expression of the vesicular glutamate transporter 1 (VGluT1) during neurulation and found that VGluT1 transcripts are present at these developmental stages. Through whole-mount immunostaining we found that VGluT1 protein localizes to medial regions of the neural plate. Knocking down VGluT1 expression by injecting either of two different targeted morpholinos or by gene deletion using Crispr-Cas9 approach increases the incidence of NTDs, indicating that VGluT1 is necessary for neural tube formation.
In vivo imaging of neurulating embryos expressing the genetically-encoded, glutamate-sensor iGluSnFR reveals that its signal is selectively brighter in the neural plate compared to the non-neural ectoderm, thereby suggesting that glutamate is released from neural plate cells. We also tested whether glutamate release is dependent on calcium entry, as in glutamatergic synapses, by using Glutamate ELISA kit. Addition of ionomycin increases glutamate released by neurulating

embryos which is significantly attenuated in embryos deficient in VGluT1. Moreover, addition of ionomycin enhances the fluorescence intensity of iGluSnFr signal in neural plate cells. In turn, released glutamate may recruit calcium dynamics in neural plate cells. We find that unilateral knockdown of VGluT1 decreases the number of spontaneous calcium transients in the affected half neural plate and impairs its folding.
Altogether these findings suggest that VGluT1 is necessary for the calcium-dependent exocytotic vesicular release of glutamate during neural plate folding which is necessary for the formation of the neural tube. Elucidating the mechanisms of neurotransmitter signaling during neurulation may contribute to identify AEDs that are safe during pregnancy.

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

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 606.26

Topic: A.07. Developmental Disorders

Support: R01MH118827
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R01MH116582
R01HD064743
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R01AG067025
U01MH116492

Title: Elevated MAP1B impairs neuronal development and social behaviors

Authors: Y. GUO, M. SHEN, Q. DONG, N. M. MENDEZ-ALBELO, J. LE, M. LI, S. X. HUANG, E. D. JAREMBOSKI, K. A. SCHOELLER, M. E. STOCKTON, C. L. SIROIS, V. L. HORNER, D. WANG, Q. CHANG, ***X. ZHAO**;
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Abstract: More than 2,000 copy number variants are associated with autism spectrum disorders (ASD), however functional impacts of affected genes are largely unknown. Here we investigated a small triplication in 5q13.2 (5q13.2trip) found in an ASD patient, encompassing only four genes. We discovered that elevated expression of the gene encoding microtubule-associated protein 1B (MAP1B) impaired development of both mouse and human neurons. Targeted activation of the endogenous *Map1b* gene in excitatory neurons of the prefrontal cortex leads to impaired social behaviors in mice. Genetic reduction of MAP1B rescues morphological and electrophysiological deficits of iPSCs-derived neurons from the 5q13.2trip ASD patient, indicating elevated MAP1B may contribute to ASD pathogenesis.

Disclosures: **Y. Guo:** None. **M. Shen:** None. **Q. Dong:** None. **N.M. Mendez-Albelo:** None. **J. Le:** None. **M. Li:** None. **S.X. Huang:** None. **E.D. Jaremboski:** None. **K.A. Schoeller:** None. **M.E. Stockton:** None. **C.L. Sirois:** None. **V.L. Horner:** None. **D. Wang:** None. **Q. Chang:** None. **X. Zhao:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.27

Topic: A.07. Developmental Disorders

Support: by KAKENHI: 15F15105, 16H06316, 16H06463, 19K16529, 21H00202, 21H04813, 21K19351
JSPS fellowship
TBRF fellowship
JST CREST: JPMJCR16G3
AMED: JP21wm0425011
Takeda Science Foundation

Title: A common pathologic progenitor cell mechanism underlying the systemic immune dysregulation in autism

Authors: *C. LIN[1,2], D. E. SEPTYANINGTRIAS[2], M. KONDA[3], K. ATARASHI[4,3], K. TAKESHITA[3], I. NIKAIDO[5,6,7], K. HONDA[4], T. J. MCHUGH[2], T. TAKUMI[1];
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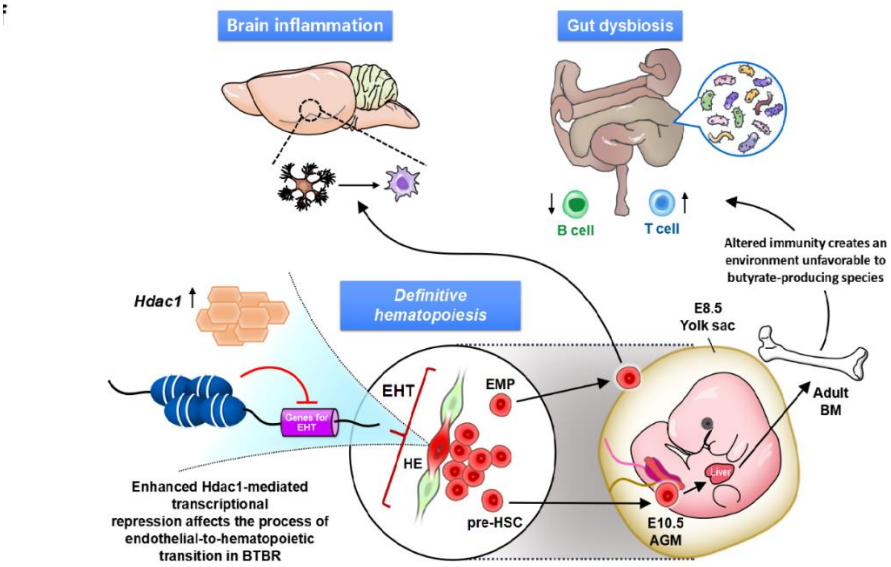
Abstract: The established role of immune dysregulation on autism etiology has emerged for over two decades. However, the molecular mechanism behind these changes remaining elusive. Considering the critical developmental windows for immune insult of autism, we suggest that tracking the origin of immune dysregulation back to embryonic stage in specific cell types should provide a rational direction to explore the underlying mechanism. In parallel, the emergence of gut-microbiota-brain axis starts another boom to pursue the role of dysbiosis in the pathogenesis of autism. However, the findings in autistic patients are often heterogenous and contradictory between studies. By using a valid mouse model of immune dysregulation, the BTBR strain, we found the increased HDAC1 activity affects the definitive hematopoiesis in yolk sac and AGM, which therefore affects the development of microglia and hematopoietic stem cells and subsequently leads to brain inflammation and skewed immune cell profiles. We also demonstrated the causality of a specific autistic immune profile with a specific pattern of dysbiosis, which reveals the potential of microbiome for the diagnosis of ASD caused by immune dysfunction. Furthermore, we identified the active endogenous retrovirus (ERV) during

embryonic development works at the most upstream to predominate the epigenetic machinery. The analogy between cellular responses induced by ERV and exogenous viral infection again echoes the etiology of epigenetic perturbation in the autism models of environmental risk factor, such as maternal immune activation (MIA) and valproic acid (VPA)-induced models of autism. With the new advance in the old model, our study unravels the idiopathic etiology of BTBR strain, which not only provides evidence for the origins of immune dysregulation in autism, but also provide new insights to how the ancient viral infection affects autism susceptibility.



Disclosures: **C. Lin:** None. **D.E. Septyaningtrias:** None. **M. Konda:** None. **K. Atarashi:** None. **K. Takeshita:** None. **I. Nikaido:** None. **K. Honda:** None. **T.J. McHugh:** None. **T. Takumi:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Program #/Poster #: 606.28

Topic: A.07. Developmental Disorders

Support: FAPESP 2019/09207-3
CAPES - Finance Code 001
CAPES No: Q6 88887.374230/2019-00
CNPq 304566/2019-5
FAPESP 2020/01107-7
FAPESP 2022/00527-8

Title: Ndel1 activity modulation due to adjunctive effects of sodium nitroprusside on symptoms amelioration in a blind-randomized clinical evaluation of patients with schizophrenia and animal model

Authors: *M. HAYASHI[1], J. NANI[2], J. E. C. HALLAK[3];
[1]Unifesp, Sao Paulo, Brazil; [2]unifesp, SAO PAULO, Brazil; [3]usp-rp, ribeirao preto, Brazil

Abstract: Schizophrenia (SCZ) is a multi-factorial and chronic disease that affects about one percent of the world population. The etiology and pathophysiology of this complex brain disorder remain unknown, contributing for the limitation in therapeutic choices, in which the pharmacotherapy is mainly centered on the modulation of monoamine neurotransmitters by employing antipsychotics. We have demonstrated significant lower nuclear distribution element-like 1 (Ndel1) oligopeptidase activity in first episode psychosis and chronic SCZ, with even lower activity in treatment resistant SCZ. Several evidences highlighting Ndel1 S-nitrosylation or decreases in Ndel1 activity as a key activity-dependent mechanism underlying the neurodevelopmental abnormalities and impairments in prefrontal cortex functioning have been reported. Translational clinical trials designed to evaluate the effects of the NO donor sodium nitroprusside (sNP) in acute and chronic SCZ, including treatment resistant, have demonstrated the improvement of SCZ symptoms following sNP infusion, in patients already taking antipsychotics. The involvement of NO in different aspects of SCZ, including in the neurobiology, cortical neurons maturation and viable synaptic connections formation, which are also in accordance with the neurodevelopmental hypothesis of SCZ, is recognized. Aiming to conciliate these independent studies showing the involvement of decreased Ndel1 activity with the neurodevelopmental hypothesis of SCZ and the more recent demonstration of the association of this activity with the clinical symptom amelioration in SCZ, we evaluated here the Ndel1 enzyme activity in a double-blinded randomized clinical cohort of patients with chronic SCZ receiving sNP. We have also assessed the Ndel1 activity in a validate animal model for SCZ after the infusion of sNP or placebo. A significant reduction of psychiatric symptoms and Ndel1 activity was observed after infusion of sNP compared to placebo in a double-blind randomized clinical cohort, similarly as also observed in an animal model for SCZ. Therefore, the present data suggest a possible involvement of Ndel1 in the mechanisms underlying the symptoms improvements of medicated SCZ patients following the sNP infusions. Although further studies are still necessary to clarify the exact molecular mechanism leading to the beneficial effects of the enhancement of nitrergic activity in medicated SCZ patients, the demonstration of a possible involvement of Ndel1 open new roads to better understand the mechanisms underlying the pharmacotherapeutic effects of NO donors, such as the sNP, on SCZ symptoms amelioration.

Disclosures: **M. Hayashi:** None. **J. Nani:** None. **J.E.C. Hallak:** None.

Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 606.29

Topic: A.07. Developmental Disorders

Support: CIHR
NSERC
Ontario Research Fund
Ontario Brain Institute
Canadian Foundation for Innovation
Brain Canada

Title: Host Genotype can be Reliably Predicted from Microbiome Structure and Neuroanatomy

Authors: *M. GREEN[1], S. ASBURY[1], J. LAI[2], K. C. RILETT[1], B. DARWIN[4], J. ELLEGOOD[5], J. P. LERCH[5], J. A. FOSTER[3,6,7];
[1]Psychiatry and Behavioural Neurosciences, [3]Psychiatry & Behav Neurosci, [2]McMaster Univ., Hamilton, ON, Canada; [4]Mouse Imaging Ctr., The Hosp. for Sick Children, Toronto, ON, Canada; [5]Mouse Imaging Ctr., Hosp. For Sick Children, Toronto, ON, Canada; [6]Res. Inst. at St. Joe's Hamilton, Hamilton, ON, Canada; [7]Ctr. for Depression Res. and Clin. Care, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Over a decade of recent studies have highlighted the role of the microbiome in regulating host health, engaging in bidirectional communication that modulates development of the immune, metabolic and nervous systems. However, understanding interactions between the microbiome and host genotype remains an active area of research, and the relative contribution of genetic factors to gut-brain communication remains a subject of debate. As our lab has demonstrated in mice, there are significant genotype-specific differences in both microbiome composition and behavioural profile, even among "wild type" strains housed within the same facility. This work was extended into selectively immune-deficient mice lacking functional T-cells, which display differences in behaviour, neuroanatomy and microbiome structure that persist across the lifespan. In light of this evidence, we hypothesized that integration of microbiome and neuroanatomical data would allow for reliable prediction of host genotype, based on distinct relationships between regional brain volumes and taxonomic abundance. Several previous studies have implemented machine learning (ML) algorithms to draw associations between brain structure, microbiome composition, behaviour and other phenotypic traits. Indeed, recent work from our lab decisively demonstrated that brain structure can be used to predict host genetics and behaviour with a high level of accuracy. Thus, to further test our hypothesis, we sought apply and extend this approach to a large existing brain volume (BV) and 16S rRNA sequencing dataset of over 450 mice of 6 different genetic backgrounds. Random forest (RF) modelling and unsupervised hierarchical clustering (UHC) were used to construct a gut microbiota-brain map that reliably distinguishes mice of differing immune status and genetic background. Our results show that both microbiome and BV data can predict genotype of the host animal with high accuracy (>95%) across all classes, as well as very high micro (>0.98) and macro (0.99) F1 scores. Furthermore, using a feature scoring in combination with recursive feature elimination (RFE), we were able to determine a small subset of mixed features that predicted genotype with a higher accuracy (>97%) than microbiome or BV data alone. Finally, UHC based on 54 select microbiome/BV features (< 20% of original variables) robustly categorized animals into 6 genotype classes, in high agreement with true genotype (>92%). Taken together, these results show that reduced-dimensionality 16S and BV data can together

provide a robust, genotype specific signature of the gut-brain axis, with potential functional implications for host behaviour.

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Poster

606. Molecular Mechanisms Underlying Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 606.30

Topic: A.07. Developmental Disorders

Title: Ifn-inducible double-stranded rna dependent inhibitor and repressor of p58 (prkrir) interacts with sars-cov-2 proteins to regulate signaling pathways

Authors: *N. BASAZINEW, M. MOSHI, M. GHANI, C. CHANG, E. OH, V. VO;
Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: PRKRIR is a negative regulator of PKR inhibitor and a RIG-I interacting protein that inhibits virus replication in the infected host. In a proteome-wide genetic investigation, we identified multiple host protein targets, such as PRKRIR that bind to the SARS-CoV-2 non-structural proteins. Here, we investigate how mutations in PRKRIR may cause neurological phenotypes associated with COVID-19. We will characterize the phenotypes associated with PRKRIR loss of function mutation, explain the mechanisms of how the disease manifests, and propose a potential therapeutic option to rescue the function of the mutant protein. To do so, we will breed and genotype CRISPR Cas9 knockout mice, and use histology techniques to study the function of PRKRIR in health versus in a diseased state.

Disclosures: N. Basazinew: None. **M. Moshi:** None. **M. Ghani:** None. **C. Chang:** None. **E. Oh:** None. **V. Vo:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.01

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

Title: Scalable and Longitudinal Video-Based Infant Digital Tracking for Earlier Detection of Neurodevelopmental Derail

Authors: *R. RAI, E. TORRES;
Psychology, Rutgers Univ., Piscataway, NJ

Abstract: In recent years, the rates of neurodevelopmental disorders such as autism, ADHD, and movement/learning disabilities have increased in children of school age. Current criteria detect these disorders on average by 4.3 years of age because they depend on maturation of social and emotional parameters detectable at present by observation alone. Such reliance on observation restricts the description of movements to those that are less ambiguous to the naked eye, focusing primarily on goal-directed motions. This approach leaves out important spontaneous components of the continuous stream of motions that make up natural behaviors since birth. Indeed, movements that occur spontaneously and lend fluidity to social behavior occur largely beneath awareness and escape the naked eye of the observer. Furthermore, they are present since birth, through various central pattern generators (CPGs), unlike the more reliable social movements that appear and mature later in life, as the neocortex further develops, and the infant's motor systems mature. In this work, we take the approach of examining the continuous stream of movements, both goal-directed, under precise top-down precision control, and bottom-up spontaneous movements, likely connected to CPGs. To that end, we use off-the-shelf, commercially available smart phones to collect video at home, collaboratively with the parents. We track longitudinal data sets obtained from two babies since birth, across 17 weeks. Furthermore, we augment our data with 7 cross-sectional sets of videos from open sources, spanning ages from 10 days to 4 months of life. We use OpenPose and extract 25 body joints with confidence criteria to select the cleanest data. For the first time, we divide the body into proximal, articulated, and distal joints, to digitally track their very early development of neuromotor control. As movements transitioned from random-spontaneous to predictive-intentional, we uncovered an orderly maturation trajectory whereby proximal joints (head, neck, shoulders, trunk) stabilized earlier than articulated joints, which in turn, preceded stabilization of end effectors. We here offer new insights into the neurotypical trajectory of kinematic synergies that simplify neuromotor control by the developing brain. Our work provides proof of concept for a new model that digitizes neurodevelopment from birth, characterizes with high precision, typical population patterns of maturation and does so collaboratively with families. The new model is deployable at scale and poised to advance the study of neuromotor control in neonates, leading to much earlier detection of neurodevelopmental derail.

Disclosures: **R. Rai:** None. **E. Torres:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.02

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

Support: NJ Governor's Council for Autism # CAUTI18ACE15

Title: Bibliometric Analyses of Observational vs. Digital Means of Screening Infant Neurodevelopment

Authors: *H. VARKEY[1], H. PHAN[2], A. GORDON[2], P. KITTLER[2], E. TORRES[1];
[1]Rutgers Univ. Dept. of Psychology, Piscataway, NJ; [2]New York State Inst. for Basic Res. in Developmental Disabilities, Staten Island, NY

Abstract: Neurodevelopmental disorders are on the rise, but the average age of detection is 4.5 years old. This delay is partly due to reliance on social-communication criteria. These require a level of maturation that takes much longer than scaffolding elements of neuromotor control. These earlier components of neurodevelopment include early evolution of reflexes, development of interactions between central pattern generators and cortical structures, maturation of intentional movements and their overall sensation. Such elements have been studied in General Movement Assessment (GMA) [1] using observational means, but the last two decades have seen a surge in digital tools (biosensors, video-based pose estimation and algorithms) that permit non-invasive tracking of newborns' movements. Despite their importance, these tools are not yet broadly used. In this work, using VOSViewer software [2], we investigate the evolution of the literature on GMA and the methods in use today, to estimate how digital techniques are being adopted. To that end, we created maps of key word co-occurrences and co-authors of 816 publications based on a search in the Web of Science database for: 'general movements' OR 'general movement assessment' OR 'general movements assessment'. The nodes on the maps were categorized by cluster groups and year of publication. Further, the methods of the general movements' literature were compiled and analyzed with a term co-occurrence map based on text data. We found that the state-of-the-art methodology to diagnose neurodevelopmental disorders still relies heavily on observation. Several groups in classical GMA research have branched out to incorporate new techniques, but few groups have adopted digital means. We report on additional analyses of methods and biosensors usage, and propose that combining traditional clinical observation criteria with digital means may allow earlier diagnoses and interventive therapies for infants.
[1] Prechtl, H. (2001). General movement assessment as a method of developmental neurology: New paradigms and their consequences The 1999 Ronnie MacKeith Lecture. *Developmental Medicine & Child Neurology, 43*(12), 836-842.
[2] Van Eck, N.J., & Waltman, L. (2007). VOS: a new method for visualizing similarities between objects. In H.-J. Lenz, & R. Decker (Eds.), *Advances in Data Analysis: Proceedings of the 30th Annual Conference of the German Classification Society* (pp. 299-306). Springer.

Disclosures: H. Varkey: None. **H. Phan:** None. **A. Gordon:** None. **P. Kittler:** None. **E. Torres:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.03

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

Title: Interpretable Digital Biomarkers as a New Model of Data Acquisition and Analyses

Authors: *J. VERO[1], E. B. TORRES[2];
[1]Rutgers Univ., Rutgers Univ., Matawan, NJ; [2]Psychology Dept, Rutgers Univ. Dept. of Psychology, Piscataway, NJ

Abstract: Clinical screening tools, such as the Montreal Cognitive Assessment (MoCA), the Autism Diagnosis Observation Schedule (ADOS) and the Unified Parkinson's Disease Rating Scale (UPDRS) are accessible, low-cost, and relatively easy-to-administer screening tests for cognitive, social, and motor (CSM) impairments, with high sensitivity and specificity (1-3). Most of these paper-and-pencil tests are structured into sub-assessments that focus on specific attributes of CSM performance. Notwithstanding their utility, a major downside of these assessments is that they are typically based on discrete scores drawn from observation, that have not been broadly mapped across the population, onto continuous streams of physiological data underlying natural behaviors (4, 5). This restriction misses the opportunity to objectively glean insight into the root cause of the deficit, only highlighting that such a deficit exists. To address this need, we here introduce the use of ON- and OFF-body consumer-grade sensors integrated with apps that leverage multimodal data. Pairing continuous digital data streams with such well-defined clinical criteria, we advance the notion of interpretable digital biomarkers. This hybrid clinical and digital model can scale the number of participants, diversify the data across the population, increase the frequency of data collection/participant, and enable longitudinal sets with fewer trips to an office/lab, for longer-term engagement and retention of participants (6). References1.Z. S. Nasreddine et al., The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 53, 695-699 (2005).2.C. Lord et al., The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30, 205-223 (2000).3.S. Fahn, R. Elton, in Recent Developments in Parkinson's Disease, Fahn S., C. D. Marsden, D. B. Calne, M. Goldstein, Eds. (Macmillan Health Care Information, Florham Park, NJ, 1987), vol. 2, pp. 15 13-163, 293-304.4.J. Ryu, J. Vero, R. D. Dobkin, E. B. Torres, Dynamic Digital Biomarkers of Motor and Cognitive Function in Parkinson's Disease. J Vis Exp, (2019).5.H. Bokadia, R. Rai, E. B. Torres, Digitized Autism Observation Diagnostic Schedule: Social Interactions beyond the Limits of the Naked Eye. J Pers Med 10, (2020).6.K. J. Steinman, W. L. Stone, L. V. Ibanez, S. M. Attar, Reducing Barriers to Autism Screening in Community Primary Care: A Pragmatic Trial Using Web-Based Screening. Acad Pediatr 22, 263-270 (2022).

Disclosures: J. Vero: None. **E.B. Torres:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.04

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

Support: CAUT14APL018

Title: Cardiac Responses to Pain as Clinically Interpretable Biomarkers for Pain-Induced Stress in Autism Spectrum Disorder

Authors: *M. ELSAYED[1], E. B. TORRES[2];
[1]Rutgers Univ., Rutgers Univ., Piscataway, NJ; [2]Rutgers Univ. Dept. of Psychology, Rutgers Univ. Dept. of Psychology, Piscataway, NJ

Abstract: Pain sensation often goes unnoticed in nonverbal individuals such as those with Autism Spectrum Disorder (ASD). Current pain assessments rely on rating scales and questionnaires which assume a capacity to understand and verbalize mental/emotional states. Behavioral observation from parents and clinicians is often subject to limitations and misinterpretation which in turn influences quality of care and delays appropriate diagnosis and treatment. The goal of this exploratory work is to uncover biomarkers of pain by investigating cardiac signals from the autonomic nervous systems (ANS). We target the neurotypical (NT) population to understand signature responses to pain that can be used to objectively assess when those with physical pain and/or neurodevelopmental disorders demonstrate such responses under normal conditions. NT subjects performed various motor-cognitive tasks such as resting, walking, and pointing to a target under control and pain conditions while ASD subjects performed the same tasks only under control conditions. Electrocardiographic (ECG) signals representing cardiac activity (via the ANS) were characterized via clinically relevant heart rate variability (HRV) metrics to assess sympathetic and parasympathetic nervous system activation along with more personalized analyses. This enables us to build clinically interpretable biomarkers of pain. Preliminary findings suggest unique statistical patterns in cardiac activity during the pain condition that are comparable to the patterns in cardiac activity observed in ASD subjects at baseline. In NT subjects, the pain condition elicits a signature cardiac response that is associated with elevated sympathetic activity (fight-or-flight response) and/or decreased parasympathetic activity (vagal tone) commonly observed in ASD subjects. Results from personalized parameterization also follow consistent patterns with time & frequency domain HRV metrics. Overall, this work has several implications in developing a clearer neurophysiological understanding of pain and can ultimately help in objectively detecting internal levels of pain and pain-related stress/anxiety in autistic and nonverbal individuals as well as the general population.

Disclosures: M. Elsayed: None. **E.B. Torres:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.05

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

Support: NJGCA CAUT18ACE014

Title: Using a common auditory test to detect neurodevelopmental derailment during neonatal stages

Authors: *E. B. TORRES[1], H. VARKEY[1], J. VERO[1], H. T. PHAN[2], P. KITTLER[2], A. GORDON[2], E. B. LONDON[2], E. A. SIMPSON[3], C. DELGADO[3], R. DELGADO[4];
[1]Rutgers Univ., Piscataway, NJ; [2]NYS Inst. for Basic Res., Staten Island, NY; [3]Univ. of Miami, Coral Gables, FL; [4]Intelligent Hearing Systems, Miami, FL

Abstract: Neurodevelopmental disorders are on the rise worldwide, with diagnoses that detect derailment from typical milestones by 3-4.5 years of age. By then, the circuitry in the brain has already reached some level of maturation that inevitably takes neurodevelopment through a different course. There is a critical need then to develop analytical methods that detect problems much earlier and identify targets for treatment. A common hearing test administered at birth—the auditory evoked brainstem response—provides estimated latencies of signal propagation and arrival through I-VII peaks, detectable along the brainstem regions, from the cochlea (peak I) to the pons (peak III), to the midbrain (peak V) and on to the primary auditory cortex. We integrate ABR data from multiple sources, including neonatal ABR from over 200K neonates at the neonatal intensive care unit and the well-baby nursery, another set of 54 neonates, among them, 31 received a clinical autism diagnosis years later, and similar ABR information for 65 young infants and children spanning 1.8-6.8 years of age, with a subset of 18 that also received an autism diagnosis. Using new methods of stochastic analyses and standardized micro-movement spike data types derived from the ABR fluctuations in amplitude and timing, we produce the earliest known digital screening biomarker to flag neurodevelopmental derailment in neonates. We discuss the new data type previously overlooked and derived from the traditional ABR responses along with the new analytical techniques that revealed the unambiguous differentiation between neonates that underwent typical development and those who went on to receive the autism diagnosis. Besides automatically separating the groups, our results revealed that the atypically developing neonates have large cumulative delays on the order of milliseconds, in latencies from peak I-VII of the ABR waveform. Furthermore, they show consistently much narrower bandwidth of latency responses at each peak. Within the context of this commonly used test, we suggest that with very little effort and cost, we can repurpose the peaks' amplitude and latency data to advance diagnoses of autism and other neurodevelopmental disorders on the rise today. We also discuss concrete targets for treatment and offer a unifying statistical approach to guide a personalized course of maturation in line with the highly nonlinear, accelerated neurodevelopmental rates of change in early infancy.

Disclosures: E.B. Torres: None. **H. Varkey:** None. **J. Vero:** None. **H.T. Phan:** None. **P. Kittler:** None. **A. Gordon:** None. **E.B. London:** None. **E.A. Simpson:** None. **C. Delgado:** None. **R. Delgado:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.06

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

Support: CAUT18ACE014

Title: New Computational Methods of Dynamic Transcriptome Interrogation to Study Neurodevelopmental Motor Disorders of the Autism Spectrum in Human and Mouse Embryonic Stem Cells

Authors: *T. BERMPERIDIS[1], S. SCHAFER[2], F. GAGE[2], T. SEJNOWSKI[3], E. TORRES[1]; [1]Psychology, Sensory Motor Integration Lab., Rutgers Univ., Highland Park, NJ; [2]Gage Lab., [3]Computat. Neurobio. Lab., Salk Inst., San Diego, CA

Abstract: Early differentiation of human embryonic stem cells (hESCs) is highly dynamic throughoutneuronal differentiation, with varying stochastic patterns of gene expression. The same gene maybe expressed or not expressed at different days of measurement/development, and/or show highor low expression variability throughout the process. Furthermore, interactions with other genesmay create statistical co-dependencies that methods like t-SNE tend to discard in thousands ofgenes. Recent work combining hESCs open-access data (*1*) with current tools from statistics andgraphical modelling, demonstrated that such low expressing, odd-varying genes may turn out toplay important roles during early stages of human neuronal differentiation (*2*). We here applythese new tools to open-access data (*3*) from mouse embryonic stem cells (mESCs) comparingstereotypical sequences of intermediate state to generate specific mature fates (standardprogramming) *vs.* methods that drive differentiation to similar mature fates by ectopicallyexpressing terminal transcription factors (direct programming.) We found that both the standardand direct programming methods reveal similar early neural commitment that later diverges intonovel transitional stages in direct programming but converges to similar final motor neuron stateas the standard path (*3*). Using our new computational tools, we first re-examined the transitionalstages to ask whether our methods recapitulate the specific and uncharacteristic gene expressionfound by (*3*), that led to direct motor neuron state. Then, we interrogate those genes associatedwith various subtypes of autism (*4, 5*)) likely expressing different dynamics than adults'progressive motor diseases.**References**1.Z. Yao *et al.*, A Single-Cell Roadmap of Lineage Bifurcation in Human ESC Models ofEmbryonic Brain Development. *Cell Stem Cell* **20**, 120-134 (2017).2.T. Bermperidis, S. Schafer, F. H. Gage, T. Sejnowski, E. B. Torres, Dynamic Interrogationof Stochastic Transcriptome Trajectories Using Disease Associated Genes Reveals DistinctOrigins of Neurological and Neuropsychiatric Disorders. *Front. Neurosci.* **16**, 1-23 (2022).3.J. A. Briggs *et al.*, Mouse embryonic stem cells can differentiate via multiple paths to thesame state. *Elife* **6**, (2017).4.E. B. Torres, Precision Autism: Genomic Stratification of Disorders Making Up the BroadSpectrum May Demystify Its "Epidemic Rates". *J Pers Med* **11**, (2021).5.S. T. Schafer *et al.*, Pathological priming causes developmental gene networkheterochronicity in autistic subject-derived neurons. *Nat Neurosci* **22**, 243-255 (2019).

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.07

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

Support: NIH 5R01NS111220

Title: Sexual dimorphism in the development of neuronal and glial populations in the dorsal root ganglia at postnatal day 0 by single-cell mass cytometry

Authors: *S. VRADENBURGH[1], A. KEELER[2], A. VAN DEUSEN[1], E. ZUNDER[3], C. DEPPMANN[3];
[1]Univ. of Virginia Neurosci. Program, Univ. of Virginia Neurosci. Program, Charlottesvile, VA; [2]Univ. of Virginia, Charlottesville, VA; [3]Univ. of Virginia, Charlottesvile, VA

Abstract: The somatosensory nervous system, which involves the sensations of touch and proprioception, is vital for interaction with our environment. The neuronal and glial cells that comprise the somatosensory nervous system are housed within a structure known as the dorsal root ganglia (DRG). Although there have been many studies that have examined how DRG neurons and glia develop and function, few studies have examined how sex may impact the development of the cell types that make up the DRG. To address this gap in knowledge, we have used mass cytometry to examine how sex impacts the maturation and development of neuronal and glial cell types within the DRG in early postnatal mouse pups. Mass cytometry is a variant of flow cytometry that uses isotopically pure rare earth metals and atomic mass spectrometry instead of fluorophores and fluorescence detection of markers on single cells. This approach allows for 40 simultaneous molecular measurements of markers pre-determined to identify distinct neuronal and glial populations and maturation states in individual cells. With this technique, we have assessed alterations in abundance of distinct cell populations within the DRG between male and female mouse pups at postnatal day 0. We found that females have a larger relative abundance of neurons when compared to males for all neuronal populations present in the DRG at this age. When examining neuronal populations more specifically, females have a larger relative abundance of TrkB+ and TrkC+ neuronal populations, while males have a greater relative abundance for peptidergic nociceptors. Although no significant differences were found in broad types of glial cells, when examining the relative abundance of specific sub-populations of Schwann cells in the DRG, significant differences in relative cell abundance exist between male and female pups. These results indicate that sex differences in the abundance of distinct cell populations in the DRG exist at postnatal day 0 for both glial and neuronal populations and can be detected by mass cytometry. Current and future findings will improve our understanding of how sex impacts neuronal and glial cell development in the DRG and shed light on why some

studies have found sex-dependent differences in sensitivity to painful tactile stimuli. Additionally, this work highlights the power and utility of mass cytometry as a technique to examine neural cells.

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.08

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

Support: NSERC Grant RGPIN-2016-04695

Title: Swimbladder development is curtailed in zebrafish embryos exposed to (-) THC

Authors: *D. ALI[1], M. AMIN[2];
[1]Biol. Sci., Univ. Alberta, Edmonton, AB, Canada; [2]Biol. Sci., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Cannabis is one of the most commonly used illicit recreational drugs and is widely used for medicinal purposes. The major psychoactive ingredient in cannabis is THC, whereas the major non-psychoactive ingredients are CBD and CBN. It is reported that up to 14% of pregnant females aged between 12-44 have used cannabis during their first trimester. The predominant form of THC in cannabis is the (-) stereoisomer. Therefore, we sought to examine the effect of (-) trans δ^9THC (0.001 mg/L to 20 mg/L) on zebrafish development. We found that zebrafish embryos exposed to (-) THC for 5 hours during the period of gastrulation experienced alterations to gross morphology, cardiac activity, MN branching and locomotion, which was similar to the effects of other cannabinoids we have tested. Importantly, (-) THC (>0.5 mg/L)-treated zebrafish did not survive past 15 days of development. Since embryos treated with (-) THC remained at the bottom of the larval dishes and did not rise in the water column, we investigated swimbladder development and found that the swimbladders of treated fish were not properly developed. Block of CB1 or CB2 receptors with AM 251 or AM 630 respectively did not prevent the effects of (-) THC. Similarly, block of TRP channels with AMG9090 did not prevent the effects of (-) THC. However, co-application of the sonic hedgehog activator, Purmorphamine with (-) THC partially prevented the effects of (-) THC alone, suggesting that the sonic hedgehog pathway may be involved in the effects of (-) THC. In support of this, RNAseq analysis revealed the downregulation of hedgehog genes, including *ptch2*, *smo*, *gli1* and *gili2b*. following treatment with (-) THC. These findings suggest that (-) THC may alter swimbladder development via the sonic hedgehog pathway.

Disclosures: D. Ali: None. **M. Amin:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.09

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

Support: NIH Grant R01NS108424-03S1

Title: From meme to gene: how social experience affects cell-type development early in life

Authors: *C. G. OROZCO, G. KONOPKA, T. F. ROBERTS;
Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Humans and songbirds, like zebra finches, transition through a sensitive period during which they can learn their species typical vocalizations (speech or song). Sensitive periods limit when experience can influence the development and function of neural circuits. Experience with adult models is thought to be necessary for shaping the development of neural circuits required for vocal learning. HVC (acronym used as proper name) is a forebrain region necessary for birds to form a memory of their fathers' song. Because zebra finches are altricial, they form lifelong memories of their fathers' song while the different classes of projection neurons in HVC are still undergoing rapid development. We hypothesized that differences in the development of different classes of neurons and their gene expression patterns underlie transitions through the developmental sensitive period. To examine this idea, we conducted single-nuclei RNA sequencing (snRNA-seq) of all HVC cell-types at different time points during the sensory learning period and compared it to adult HVC. We found that most of the mature projection neurons in adults project to the song motor region RA (robust nucleus of the arcopallium). Conversely, most of the mature projection neurons in juveniles project to the auditory region Av (nucleus avalanche). To test if these changes were experience-dependent, we compared cell-types from juvenile zebra finches' that had not received any experience with a song tutor with birds receiving 2 days of tutoring experience. We found that tutoring experience increased the proportion of mature neurons projecting to the auditory forebrain. Together, these results highlight the role of experience in the maturation of cell-types in HVC and highlight the role of a little studied auditory-projecting neuron during the early stages of vocal learning.

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.10

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

Support: NIH Grant R01DC016696
Stowers Funding

Title: The Wnt receptor Frizzled1 interacts with odorant receptors and controls rewiring plasticity of olfactory sensory neurons during the critical period

Authors: *J. LEE[1], Y. WU[1], L. MA[1], S. YI[2], R. HERVAS[2], Y. ZHANG[1], Z. WEN[1], L. FLORENS[1], R. YU[1,3];
[1]Stowers Inst. for Med. Res., Kansas City, MO; [2]Univ. of Hongkong, Pokfulam, Hong Kong; [3]Dept. of Anat. and Cell Biol., Univ. of Kansas Med. Ctr., Kansas city, KS

Abstract: An olfactory sensory neuron (OSN) expresses only one type of functional odorant receptor (OR). OSNs expressing the same OR converge their axons to a glomerulus forming the olfactory map, a structural basis for olfactory sensation. Accurate OSN connectivity must be formed for precise neuronal functionality during the critical period early in life. OSN projection is guided by molecules of which expression is regulated by OR signaling activity specific to a given OR. We previously found that the Wnt signaling receptor Frizzled1 (Fzd1) is required for the plasticity of OSN rewiring during the critical period. Both Fzd1 and OR are G protein coupled receptors (GPCRs) and GPCRs have been shown to interact and modulate each other's signaling. We hypothesized that Fzd1 interacts with ORs during the critical period and regulate rewiring plasticity by regulating OR signaling. To test this, we employed live FRET imaging and immunoprecipitation in Neuro-2a cells. Accordingly, we found that Fzd1 interacts with distinct ORs with differential affinities. Employing AlphaFold2, we modeled the dimer and tetramer of Fzd1 and ORs and identified the potential interaction sites on Fzd1. Interestingly, site-directed mutation of Fzd1 resulted in loss of interaction with only one of the tested ORs. These results suggest that Fzd1 may employ differential binding modes with distinct affinities against discrete ORs. Furthermore, Fzd1 in mice OSNs displayed interaction with ORs revealed by APEX2 proximity labeling. Strikingly, tandem mass tagged proximity labeling revealed the interaction increased upon the Wnt5a activation. These results suggest the unprecedented scenario where Wnt pathways regulate sensory receptors' activity via direct interaction of the receptors. Taken together, we propose that the Wnt receptor Fzd1 modulates OR activities and confer distinct rewiring of the OSNs. Fzd1 incurring additional dimension of OSN dynamics could govern the heightened plasticity during the critical period and proper olfactory map formation.

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.11

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

Support: NIH Grant EY021580
NIH Grant EY027407

Title: Critical period plasticity exchanges neurons active in visual circuitry

Authors: *T. BROWN, A. W. MCGEE;
Univ. of Louisville, Louisville, KY

Abstract: Abnormal visual experience during a developmental critical period can yield enduring deficits in visual function. Yet how experience-dependent plasticity alters the properties of individual neurons and composition of visual circuitry are unclear. Here we measured with calcium imaging how monocular deprivation during the critical period disrupts binocularity for thousands of neurons in visual cortex. Tracking the tuning properties of nearly a thousand neurons revealed that abnormal vision interconverts monocular and binocular neurons to alter eye dominance through the ratio of monocular neurons. In addition, some neurons more responsive to the deprived eye were silenced and previously unresponsive neurons were recruited. Thus, plasticity during the critical period adapts to recent experience by both altering the tuning properties of responsive neurons and exchanging neurons active in visual circuitry.

Disclosures: T. Brown: None. **A.W. McGee:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.12

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

Support: ANR-19-FRAL-0007-01

Title: Brain and behavioral development of distractibility during childhood: an EEG study

Authors: *A. BIDET-CAULET[1,2], P. ALBOUY[3], R. HOYER[3,1];
[1]Lyon Neurosci. Res. Ctr., Lyon, France; [2]Inst. de Neurosciences des Systèmes, Marseille, France; [3]Univ. Laval, Ctr. de recherche Univ. Laval, Québec, QC, Canada

Abstract: Distractibility is the propensity to behaviorally react to irrelevant information. It relies on a balance between voluntary and involuntary attention. Voluntary attention enables performing an ongoing task efficiently over time by selecting relevant information and inhibiting irrelevant stimuli, whereas involuntary attention is captured by an unexpected salient stimulus, leading to distraction. At the brain level, voluntary and involuntary attention rely on partially overlapping dorsal and ventral brain networks, respectively, which undergo significant development during childhood. The developmental trajectory of distractibility has been behaviorally characterized using a recently developed paradigm, the Competitive Attention Test

(CAT). In young children, increased distractibility was found to mostly result from reduced sustained attention and enhanced distraction, while mainly resulting from decreased motor control and increased impulsivity in teenagers. However, it is not yet clear how these behavioral developmental changes are implemented in the brain.
To address this question, we recorded electrophysiological (EEG) signal and behavioral responses from 3 age groups (6-7,11-13 and 18-25-years-old; N=45) performing the CAT. To assess voluntary attention orienting, the CAT includes informative and uninformative visual cues respectively indicating—or not—the spatial location of a forthcoming auditory target to be detected. To measure distraction, the CAT comprises trials with a task-irrelevant complex sound preceding the target sound. Moreover, the rates of different types of false alarms, late and missed responses provide behavioral measures of sustained attention, impulsivity, and motor control. EEG brain responses to relevant and irrelevant auditory events occurring during the CAT. The CNV before the target was found modulated by the cue information in adults only, in line with a cue effect on reaction times in adults only. In response to the target, a cue effect was found on the N1 in teenagers and on the P3b in children. These effects of voluntary orienting at different moments of the task according to age suggest a shift from a reactive to a proactive strategy from 6-years-old to adulthood. In response to the irrelevant sounds, a larger and longer RON was found in children and teenagers compared to adults, suggesting difficulties in reorienting back to the task before adulthood. These brain changes during childhood are associated with a behavioral increase in sustained attention, and a decrease in distraction and impulsivity. These findings give important insights into how the developing brain shapes child behavior.

Disclosures: **A. Bidet-Caulet:** None. **P. Albouy:** None. **R. Hoyer:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.13

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

Support: NIH Grant EY030458
NIH Grant EY030458 02S1

Title: The serine/threonine kinase LKB1 is required for retinal ganglion cell migration and viability.

Authors: *R. MACKIN[1], C. BURGER[2], M. A. SAMUEL[3];
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Abstract: Restoring vision using regenerative medicine is a primary goal of the visual research community. One promising method that has recently garnered intense focus is the transplantation of human induced pluripotent stem cell (iPSC) derived retinal ganglion cells (RGCs) into

diseased or degenerated retinas. However, for this to be a viable strategy the donor cells need to properly migrate and integrate into the host retinal circuitry. The molecular mechanisms required to achieve these two feats are not fully understood. Here, we demonstrate that the serine/threonine kinase LKB1 is required during the development of the murine retina for successful migration of retinal ganglion cells and maintenance of cellular viability. In cross-sections of retinas deficient in LKB1, some RGCs are observed to be "stuck" in mid transit from their point of origin at the apical surface of the retina on their way to the ganglion cell layer (GCL). By the second post-natal week of development, we observe a significant decrease in the population of RGCs. This decrease in the number of RGCs can most likely be attributed to failure to migrate to the GCL and possibly failure to successfully integrate into retinal circuitry even if successful migration has been achieved. Further investigation is required to determine if this phenotype is confined to a particular subset of RGCs or affects the population as a whole. The downstream effectors from LKB1 have also not been elucidated. Despite these knowledge gaps, our results indicate that LKB1 signaling is required to accomplish successful migration of RGCs and maintain cellular viability in development and may be an amenable target for developing improved transplantation strategies of retinal ganglion cells into diseased and degenerating retinas.

Disclosures: R. Mackin: None. **C. Burger:** None. **M.A. Samuel:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.14

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

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Title: Tgfbr2-regulated paracrine signals from dental pulp cells guide neurite outgrowth in developing teeth

Authors: M. STANWICK[1], C. BARKLEY[2], R. SERRA[2], A. KRUGGEL[1], A. WEBB[3], Y. ZHAO[3], M. PIETRZAK[3], C. ASHMAN[1], A. STAATS[1], S. SHAHID[1], ***S. PETERS**[1];
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Abstract: Transforming growth factor β (TGFβ) signals play a crucial role in tooth mineralization and innervation. During postnatal development, dental pulp (DP) mesenchymal cells secrete neurotrophic factors that guide sensory axons into and throughout the DP concurrent with dentin secretion and mineralization. We established a mouse model to conditionally delete TGFβ receptor 2 (*Tgfbr2)* in the DP mesenchyme using an Osterix promoter-driven Cre recombinase(*Tgfbr2*^{cko}). These mice developed significant defects in bones and teeth, including reduced mineralization and short roots. We performed mRNA-Sequence and gene onotology analyses using RNA from the DP of P7 control and mutant mice to investigate the pathways involved in*Tgfbr2*-mediated tooth development. These analyses identified downregulation of several mineralization-related and neuronal genes in the *Tgfbr2*^{cko} DP compared to controls. Select gene expression patterns were confirmed by quantitative real-time PCR and immunofluorescence imaging. Hematoxylin and eosin staining revealed reduced axon-like structures in the mutant mice. Reporter imaging confirmed that Osterix-Cre activity within the tooth was isolated to the DP mesenchyme. Immunofluorescence staining for the neuronal marker, β3 tubulin, was performed on control and mutant molars on postnatal days 7 and 24. Confocal imaging and pixel quantification demonstrated reduced innervation in *Tgfbr2*^{cko} first molars at both stages compared to controls. This indicated that *Tgfbr2* regulated the necessary signals in the DP mesenchyme to guide neurite outgrowth in the developing teeth. Lastly, we performed a co-culture assay with dispersed trigeminal neurons atop Transwell filters overlying primary *Tgfbr2*$^{f/f}$ DP cells. *Tgfbr2* was deleted in the DP cells using adenovirus-expressed Cre recombinase. Confocal images demonstrated increased neurite outgrowth when the neurons were cultured with *Tgfbr2*-positiveDP cells compared to neurons cultured alone. Axon outgrowth was decreased when *Tgfbr2* was deleted in the DP cells. Media conditioned during co-culture was collected for proteomics analysis to identify paracrine signals regulating axonal outgrowth. Both our proteomics and RNA-Seq analyses indicated that axonal guidance cues, particularly semaphorin signaling, were disrupted by *Tgfbr2* deletion in the DP mesenchyme. These results were published and are available at doi:10.3389/fcell.2022.834815. Follow-up studies into semaphorin signals regulating neurite outgrowth in developing teeth are in progress.

Disclosures: **M. Stanwick:** None. **C. Barkley:** None. **R. Serra:** None. **A. Kruggel:** None. **A. Webb:** None. **Y. Zhao:** None. **M. Pietrzak:** None. **C. Ashman:** None. **A. Staats:** None. **S. Shahid:** None. **S. Peters:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.15

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

Support: F32EY032756
R01EY028219
R01MH126351

Title: The synaptic basis for orientation matching in binocular visual cortical circuits

Authors: *K. TSIMRING[1], K. R. JENKS[1], J. P. K. IP[2], M. SUR[1];
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Abstract: Experience-dependent plasticity refines sensory circuits during critical periods in development. In the binocular visual cortex (bV1), visual experience aligns neuronal orientating tuning from the ipsilateral and contralateral eyes during the critical period for ocular dominance plasticity (~p22-p32). However, it remains unclear whether the alignment of somatic orientation tuning is coupled with changes at the synaptic level and through what forms of plasticity these synaptic changes take place. We propose that Hebbian and heterosynaptic plasticity shape visual responses of bV1 neurons during the critical period by strengthening synapses based on their correlation to the postsynaptic neuron and to synaptic neighbors, respectively. To examine how visual properties of bV1 neurons and their inputs change across development, we used *in vivo* two-photon calcium imaging to chronically track eye-specific and binocular visually driven responses of neurons and their dendritic spines. We find that ipsilateral eye driven responses become aligned to the soma's binocular preference over development, whereas contralateral eye driven responses are already matched at the start of the critical period. This indicates that Hebbian plasticity is at least partially responsible for the alignment of ipsilateral responses during the critical period. At the synaptic level, there is turnover of over half of the existing dendritic spines between p22 and p32. Of the dendritic spines that are retained, about a third undergo changes in their functional responses. Intriguingly, the responses of these stable spines become more aligned to the soma by the end of the critical period, further suggesting that correlated pre- and post-synaptic neurons strengthen their connectivity through Hebbian-like mechanisms to converge to a similar orientation preference. Heterosynaptic plasticity, on the other hand, may play a role in gating the output of a neuron's visual response, as we find that neighboring spines (< 5 um apart) are more correlated in responsive than unresponsive neurons. To test the contribution of Hebbian and heterosynaptic plasticity to the development of binocular matching, we are currently building a biophysical model that incorporates the structural and functional properties of dendritic spines imaged *in* vivo to simulate their dynamics between p22 and p32. By comparing the final state of synapses in the model with our experimental data at p32, we can assess which plasticity rules are necessary and sufficient to explain our results. Our work will thus provide critical insight into the nature and mechanisms of experience-dependent plasticity during development.

Disclosures: K. Tsimring: None. **K.R. Jenks:** None. **J.P.K. Ip:** None. **M. Sur:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.16

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

Support: Lundbeckfonden

Title: Postnatal Development of Hearing in Zebra Finches

Authors: *T. ANTTONEN[1], J. CHRISTENSEN-DALSGAARD[3], C. P. ELEMANS[2];
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Abstract: Zebra finch song acquisition is widely used as a research model for human infant speech development. Juvenile zebra finch males learn to sing by imitating conspecific males. The hearing sensitivity of zebra finches is believed to develop largely postembryonically, reaching adult-like frequency sensitivity around 20 days post hatch (DPH). This is shortly followed by the onset of the sensory acquisition period of song template around 20-to-30 days. Interestingly, high frequency (~8 kHz) incubation call-dependent parent-embryo communication has been recently demonstrated to affect postnatal development of the offspring. However, the physiological basis for this communication is conflicting with the known hearing capabilities of 20 DPH zebra finches. To quantify the early development of hearing in zebra finches, we have recorded air- (AC) and -bone conduction- (BC) induced auditory brainstem responses (ABRs) from juvenile zebra finches from 2-25 DPH. Our results indicate that earliest AC-ABRs can be recorded with intense broadband clicks around 4-to-6 days after hatching. The sensitivity for AC- and BC-based click and tone burst stimuli increases from 250 Hz to 6 kHz over the three first weeks from hatching and remains very limited for stimuli above 6 kHz. Interestingly, the ABR wave structure continues to develop past 20 DPH, which suggests that the zebra finch auditory pathway continues to develop during the sensory acquisition period of song learning. These results show that the early hearing function of developing zebra finches may support low frequency acoustic communication, but that it is unlikely that the hearing capability of zebra finch embryos supports high frequency parent-embryo acoustic communication.

Disclosures: T. Anttonen: None. **J. Christensen-Dalsgaard:** None. **C.P. Elemans:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.17

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

Support: NIH Grant EY006821

Title: The rapid emergence of functional modular networks in developing tree shrew visual cortex

Authors: *A. GRIBIZIS[1], D. FITZPATRICK[2];
[1]Max Planck Florida, Jupiter, FL; [2]Max Planck Florida Inst., Max Planck Florida Inst., Jupiter, FL

Abstract: In the primary visual cortex (V1) of many mammals, orientation preference is organized in a periodic pattern across cortical space known as orientation maps. Patterned spontaneous cortical activity immediately before eye opening in V1 resembles the modular topology of orientation maps, consistent with an experience-independent origin of this fundamental modular structure (Smith et al, 2018). However, the sequence of events that leads to the initial emergence of modular network structure early in development remains unclear.
Here we explore this issue in a novel model system: the developing visual cortex of the tree shrew. The mature visual cortex of the tree shrew has long served as a model for studying the functional organization of circuits in V1 due to its well-defined modular representation of visual properties and resemblance to primate V1. By taking advantage of the fact that tree shrews have a short gestation, are born altricial, 2-3 weeks before eye opening, and feed only once in a 24 hour period, we have been able to develop a chronic imaging preparation to visualize changes in patterns of V1 spontaneous activity over several days early in development.
Using viral expression of GCaMP to measure calcium signals, we find that the earliest patterns of spontaneous activity evident several days prior to eye opening have the appearance of large, isolated single patches. Following these early coarse solitary patterns of activity, distinctly finer-scale modular network patterns of activity suddenly emerge, with coactivation of multiple patches extending millimeters across the cortical surface--patterns not evident the previous day. Ongoing studies using multiphoton functional imaging, laminar electrophysiological probes, and structural analyses of cell morphology are evaluating structural changes that could be developing concurrently with this rapid emergence of modular networks.

Disclosures: **A. Gribizis:** None. **D. Fitzpatrick:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.18

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

Support: NIGMS/NIH COBRE grant #5P20GM121310-03
NIGMS/NIH P20GM103432

Title: Development of the Xenopus tadpole retinotegmental projection

Authors: U. G. UDOH, ***K. G. PRATT**;
Univ. of Wyoming Grad. Program In Neurosci., Laramie, WY

Abstract: How neurons self-assemble into circuits that give rise to behaviors is a fundamental question in neuroscience. The Xenopus tadpole retinotectal projection - the synapse between the retinal ganglion cells (RGCs) in the eye and the midbrain optic tectum - has served as a popular model to study this question. But the retinotectal projection is only one of several components of the vertebrate visual system. Previously we found that the innate preference for mid-spectrum

("green") wavelengths of light displayed by Xenopus tadpoles does not require the optic tectum but does require the tegmentum, a region of the midbrain that lies ventral to the optic tectum. Through additional anatomical and electrophysiological studies, we identified a retinotegmental projection, a direct projection from the RGCs to the midbrain tegmental neurons. In this study, we compare, side-by-side, the functional development of the retinotectal and retinotegmental projections. Whole cell electrophysiological recordings from tectal and tegmental neurons were carried out using an isolated brain preparation. Recordings were carried out at three key stages of retinotectal development: stage 42 (approximately 5 days post-fertilization; dpf) shortly after the RGC axons have entered the optic tectum and have begun to form synapses onto tectal neurons, stage44-46 (7-9 dpf) characterized by dynamic synapse formation and loss, and stage 48/49 (12-21 dpf) characterized by a more refined retinotectal projection. We found that the maximum strength of RGC input to the optic tectum peaks during stage 44-46 (pre-refinement), then declines by stage 48/49 - the more refined stage. The RGC inputs to the neurons residing in the tegmental region of the midbrain, however, did not display this transient peak. Instead, the strength of the RGC input remained stable across development, suggesting that the retinotegmental projection does not refine. Furthermore, while tectal neurons displayed a previously described homeostatic plasticity of intrinsic excitability (the ability to fire action potentials) in response to rising levels of synaptic drive, tegmental neurons displayed a relatively high and non-varying level of intrinsic excitability. Overall, the results of this comparative study suggest that these two visual projections are built differently and probably carry out different functions as part of the visual system.

Disclosures: U.G. Udoh: None. **K.G. Pratt:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.19

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

Support: NIH Grant EY013574

Title: Postnatal development of cortical magnification in rhesus macaque striate cortex

Authors: J. A. KARPF[1], L. M. RENNER[1], M. M. CHERNOV[1], D. ZARAZA[1], M. NEURINGER[1], A. W. ROE[2], *R. M. FRIEDMAN[1];
[1]Neurosci., Oregon Hlth. & Sci. Univ. - ONPRC, Beaverton, OR; [2]Interdisciplinary Inst. of Neurosci. & Technol., Zhejiang Univ., Hangzhou, China

Abstract: Primate cortical retinotopy is characterized by a large representation of foveal space that decreases at peripheral eccentricities and parallels the high cone density in adult retinal fovea. While foveal cone density exhibits profound increases during postnatal development, the functional development of corresponding striate cortex (V1) remains unclear. Using intrinsic

optical imaging, we measured cortical magnification in V1 in rhesus macaques ranging in age from 2 days after birth to adulthood. For foveal and peripheral visual eccentricities, we calculated a cortical magnification factor (CMF) based on the displacement of the activation on cortex to a visual stimulus presented at different locations in visual space and population response (PR) measured as the width of cortex responding to a visual stimulus (band-limited drifting monochromatic square wave gratings). We observed less cortical magnification at peripheral than at foveal eccentricities in striate cortex with a maximum CMF of about 15-20 mm2/deg in adults. Shortly after birth, CMFs were half of adult values, and rapidly approached adult values by week 2. CMF correlated strongly with PR, which showed a similar developmental increase. In contrast, foveal cone densities increased incrementally across the first few developmental months and were still not adult-like by 6 months. Therefore, neither CMF nor PR correlated with foveal cone density. Compared to retinal development, the accelerated functional maturation of V1 measured by cortical magnification suggests that foveal cone density is not a primary driver for the postnatal cortical expansion of foveal representation of V1.

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.20

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

Support: NIH Grant DC019737

Title: Temporally shifted critical period events in left and right primary auditory cortex

Authors: *A. P. REID, D. NEOPHYTOU, H. V. OVIEDO;
Biol., The City Col. of New York, New York, NY

Abstract: Cortical areas have unique circuit motifs that may underlie specialized functionality. One such specialization is left-hemisphere vocalization processing observed in the auditory cortex (ACx) of humans, rodents and other animals. Given the prevalence of neurodevelopmental communication disorders in humans, it is critical to understand how normal ACx development leads to lateralized functionality. Here, we characterize developmental events underlying the emergence of lateralized function in circuits of the mouse primary ACx. We find differences between left and right ACx in the timing of significant developmental events marking the critical period of heightened experience-dependent plasticity in the days surrounding the onset of hearing in mice (P12-P18). During the critical period, environmental cues coincide with changing patterns of molecular and cellular activity to enact normal cortical circuit formation. A time shift or prolonged period of circuit maturation in one hemisphere could precipitate lateralized functionality due to circuit refinement in the context of different sensory

content. Our data indicate that the right ACx consistently has an earlier onset of events associated with critical period plasticity and faster maturation of cortical circuit structure, when compared to the left ACx. Using voltage sensitive dye imaging in acute auditory thalamocortical slices, we determine the laminar distribution of thalamocortical (TC) projections at different developmental time points. We find a clear progression from immature to mature patterning in the right ACx, similar to that reported previously in the left ACx. Interestingly, left ACx maturation consistently lags behind right ACx maturation in both age-matched and within-animal comparisons. In agreement with this observation, we find that the laminar distribution of both myelination and VGluT2, which specifically marks TC terminals, also show signs of maturation earlier in the right ACx. Furthermore, we find that measures of GABAergic synaptic inhibition maturation, which is linked to both the establishment and termination of the critical period, also occur earlier in the right ACx. Parvalbumin-expressing interneuron density, perineuronal net counts, and frequency of spontaneous inhibitory postsynaptic currents, are all higher in the right ACx at earlier time points. Together, our data indicate a hemisphere-dependent shift in the timing of significant events associated with critical period plasticity, with right ACx leading left ACx in a variety of indicators of circuit maturation.

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.21

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

Support: EUY22122

Title: Impact of premature visual experience on development of receptive field properties

Authors: *S. GRISWOLD[1], S. D. VAN HOOSER[2];
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Abstract: Cortical/cerebral visual impairment (CVI) is the most common cause of visual impairment in children in the developed world. CVI is defined as visual impairment occurring in the presence of a normal eye exam, or out of proportion with observed ocular pathology, and has been observed to be more common in very preterm infants than their full term counterparts. Due to advances in neonatal medicine, very preterm infants are surviving with increasing frequency, causing a concomitant increase in CVI
Given that preterm infants may have up to 8 weeks of premature visual experience, and that premature vision has recently been demonstrated to aberrantly drive receptive field development in visual cortex, we propose that early exposure to visual stimuli may increase the susceptibility of preterm infants to CVI. Here we hypothesize that prematurely opening the eyes of ferrets will aberrantly impact the development of visual acuity in visual cortex (VC) and lateral geniculate

nucleus (LGN). We have tested this hypothesis by prematurely opening one eye of ferrets at 25 days post parturition and subsequently examining the impact of premature eye opening on contrast sensitivity, direction tuning, orientation tuning, and temporal frequency tuning between P55-65. These receptive field properties were examined by recording from ipsilateral and contralateral monocular cortex while showing anesthetized animals drifting grating stimuli in which direction, spatial frequency, and contrast were covaried. Our preliminary findings show that monocular cortex corresponding to the prematurely opened eye demonstrates reduced contrast sensitivity in comparison with monocular cortex corresponding to the developmentally normal eye, suggesting that premature vision may aberrantly impact visual acuity in V1.

Disclosures: S. Griswold: None. **S.D. Van Hooser:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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

Support: NIH DC012775
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Title: Neuronal birthdate, but not motor-derived signals, organizes a functional sensorimotor circuit for gaze stabilization

Authors: *D. GOLDBLATT[1], S. HUANG[1], M. R. GREANEY[1], K. R. HAMLING[1], V. VOLETI[2], C. PEREZ CAMPOS[2], K. B. PATEL[2], W. LI[2], E. M. HILLMAN[2], M. W. BAGNALL[3], D. SCHOPPIK[4];
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Abstract: Neuronal birthdate predicts many anatomical and functional properties of individual neurons, but the complexity of most circuits has made the relationship between birthdate and circuit-level organization previously intractable. We leveraged a simple model - the larval zebrafish vertical gaze stabilization reflex - to determine how neuronal birthdate organizes a functional sensorimotor circuit. To transform vertical pitch-tilt rotations (e.g., nose-up) into corrective eye rotations (e.g., eyes-down), the vertical gaze stabilization circuit uses a precisely-organized three-neuron arc of vestibular sensory afferents, central brainstem projection neurons, and extraocular motor neurons. To determine how canonical nose-up and nose-down subtypes are specified and integrated into circuit architecture, we examined their development in space and time. We designed a vertical rotation stimulus, compatible with both two-photon and volumetric, single-objective light-sheet (SCAPE) microscopy, to differentiate cardinal subtypes

of central vestibular neurons. To correlate subtype function with development, we presented this stimulus to anatomically-birthdated neurons. We discovered that neuronal birthdate predicts both the cardinal subtype identity and somatic localization of individual vestibular neurons, and that somatic localization anticipates subtype identity. Neuronal birthdate further predicted other functional properties, such as responses to non-preferred directional rotations and to impulse rotations. Lastly, neuronal birthdate predicted subtype integration with both upstream semicircular canal inputs and downstream extraocular motor neuron outputs. Our findings demonstrate that circuit organization emerges as early as when neurons become post-mitotic and suggest a key mechanism: spatiotemporally-available molecular cues. Currently, we are investigating the functional and transcriptional contributions of motor, sensory, and extrinsic signals to vestibular circuit organization. With a genetic loss-of-function tool, we demonstrated that motor-derived signals are dispensable for central vestibular development. Our work speaks to the developmental principles that organize functional sensorimotor circuits.

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

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Program #/Poster #: 607.23

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

Support: JSPS KAKENHI 19K22878
Naito Foundation

Title: Social buffering of isolation stress by somatosensory stimulation in mouse pups

Authors: *S. YOSHIDA[1], H. FUNATO[1,2];
[1]Fac. of Med., Toho Univesity, Tokyo, Japan; [2]Wpi-iiis, Univ. of Tsukuba, Ibaraki, Japan

Abstract: Various studies, including nonhuman primates and rodents, have shown that social isolation accompanied by decreased physical contact with caregivers is highly detrimental to mammalian development (Review in Yoshida and Funato, iScience, 2021). In addition, young mammals often huddle tightly with their littermates in the nest. Here, we examined the effects of somatosensory stimulation on the stress-buffering of isolation during the postnatal development of mouse pups. We observed huddling behavior using two pups at the postnatal day (PND) 8, 10, and 16 (n > four pairs each PND from different litters). The pups were placed in a 15 cm square space with a 23℃ or 33℃ floor for 30 min. In PND8, the two pups separated without physical contact for most of the assay at 33℃ compared to 23℃ ($p = 0.021$). PND10 pups also spend longer without physical contact at 33℃ than at 23℃ ($p = 0.027$). An increase in the number of PND10 pups showing partial physical contact during the assay at 33℃ was the behavioral feature

that distinguished it from 23℃ ($p = 0.038$). There was no significant difference in the proportion of spending no physical contact between 23℃ and 33℃ at PND16 ($p = 0.18$). PND16 pups tended to keep partial contact with each other even at 33℃. Next, we examined the plasma corticosterone (CORT) levels to assess isolation stress. PND8 pups showed a low CORT level with no significant difference regardless of the floor temperature or the presence of another pup. PND10 pups tended to have lower CORT in huddled conditions with two pups than in isolation, even at 33℃ ($p = 0.076$). Our previous study using PND16 pups showed that the huddling pups with the littermates under maternal absent conditions resulted in lower CORT levels than isolated pups (Yoshida et al, Front Cell Neurosci, 2018). Preliminary experiments suggest that pups around PND16 have a longer heartbeat interval and increased parasympathetic activity due to tactile stimulation mimicking maternal licking. In mouse pups, it is suggested that the buffering mode of isolation stress becomes more complex during the second and third weeks of life, from induction by warmth alone to induction by warmth and tactile stimulation.

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Poster

607. Neurodevelopment: From Computer Models to Patients

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Program #/Poster #: 607.24

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

Title: Effects of nicotine on parvalbumin neurons in rat somatosensory cortex during the critical period

Authors: *C. HAGA, J. KOENIG, A. KELLER;
Anat. & Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Parvalbumin-expressing neurons contribute to the regulation of experience-dependent plasticity. The maturation of these neurons coincides with the end of the somatosensory critical period, around postnatal day 14 (P14) in rats. These neurons express nicotinic receptors, but their responses to nicotine across this critical period have not been explored. We use *in vitro* patch clamp electrophysiology to record evoked and spontaneous excitatory postsynaptic potentials from fast-spiking inhibitory neurons in layer IV of the primary somatosensory cortex (S1) of male and female young rats, to test the effects of acute nicotine before and after the critical period switch. Before P14, amplitudes of evoked synaptic responses in these neurons decreased following bath-applied nicotine. However, after this developmental time-point, amplitudes of evoked responses in fast-spiking interneurons are unaffected by nicotine. We are exploring the molecular pathways through which these responses are regulated across the critical period, and testing the hypothesis that these changes are related to the developmental expression profile of Ly6 family proteins, the endogenous inhibitors of nicotinic receptors.

Disclosures: C. Haga: None. **J. Koenig:** None. **A. Keller:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.25

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

Support: National Science Foundation Grant No. IOS-1921065
National Institutes of Health, NINDS R01NS118562

Title: The transcription factor broad influences wiring in the Drosophila visual system

Authors: *N. SMOLIN[1], Y. Z. KURMANGALIYEV[2], C. R. VON REYN[1,3];
[1]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA; [2]Dept. of Biol. Chem., Howard Hughes Med. Inst., Los Angeles, CA; [3]Dept. of Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Transcription factors (TFs) are known to act as a point of control for neuronal wiring patterns, repressing or activating specific combinations of cell adhesion molecules (CAMs) that then define how neurons wire to each other. Due to the cell-type specific patterning of these factors, however, the individual identities of these TFs that regulate wiring are unknown in the majority of cell populations. To investigate how neurons choose to connect to other neurons we use the model organism *Drosophila melanogaster*. The analysis of the single-cell RNA sequencing transcriptional atlas of the *Drosophila* visual system revealed highly differential expression of one transcription factor, *broad*, between two visual system cell-types, Lobula Plate Lobula Columnar neuron type 1 and 2 (LPLC1 and LPLC2). Using the UAS/GAL4 system, we were able to overexpress *broad* in LPLC2 and knock down *broad* in LPLC1, thus allowing us to study how *broad* modulates wiring decisions between these two cell-types. We found that overexpression of one *broad* isoform (*broad-z3*) in LPLC2 changed its neuronal wiring pattern in the lobula. Changes were found in layers 2, 3, 4, 5b and 6 ($p<0.05$), such that the overall wiring pattern in LPLC2 resembled the wiring pattern of LPLC1. These changes in wiring suggest differences in presynaptic inputs to LPLC2. Specifically, the dendritic arborizations found in layers 2 and 3 suggest new presynaptic inputs from T2 and T3 cells, and we hypothesize that LPLC2 may now have altered tuning to different visual stimuli. We also found that the RNAi knock down of all *broad* isoforms in LPLC1 changed its wiring pattern in the central brain. Quantification of fluorescence revealed significant ectopic axonal branching ($p<0.05$) in the glomeruli of LPLC2 for both RNAi knock down lines tested, suggesting that LPLC1 is now arborizing into that region and making novel postsynaptic connections that normally LPLC2 would make. Although transcriptional programs for neuronal wiring often involve a combination of transcription factors, we show that *broad* may individually influences wiring without additional factors. This known differential expression of *broad* between two closely related neurons makes it an ideal system for studying principles of synaptic specificity. Future works will aim to test whether these hypothesized changes in wiring are functional synaptic connections and to identify the mechanisms behind *broad* driving differential wiring.

Disclosures: **N. Smolin:** None. **Y.Z. Kurmangaliyev:** None. **C.R. von Reyn:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.26

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

Support: National Science Foundation Grant No. IOS-1921065
National Institutes of Health, NINDS R01NS118562

Title: Wiring an Escape: Competition and Refinement in a Developing Sensorimotor Circuit

Authors: *B. W. MCFARLAND[1], H. JANG[1], Y. Z. KURMANGALIYEV[2], A. NERN[3], M. J. PARISI[4], K. C. DAVIS[4], T. J. MOSCA[4], T. A. GODENSCHWEGE[5], C. R. VON REYN[1,6]; [1]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA; [2]Dept. of Biol. Chem., Howard Hughes Med. Inst., Los Angeles, CA; [3]Janelia Res. Campus, Ashburn, VA; [4]Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [5]Biol. Sci., Florida Atlantic Univ., Boca Raton, FL; [6]Dept. of Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Sensorimotor circuits receive inputs from multiple sensory neuron cell types and must integrate these inputs across space and time into appropriate behavioral responses. The function of individual neurons within these circuits is affected by the type, number, localization of input synapses, and wiring patterns primarily established over development. Due to limitations in accessibility, we still know little about how sensorimotor circuits are wired. Here, we investigate developmental wiring in a well-established, experimentally tractable sensorimotor circuit (the Giant Fiber circuit) in *Drosophila melanogaster*. We investigate circuit formation by focusing on visual projection neurons (VPNs) as they compete for space on dendrites of a shared sensorimotor neuron, the Giant Fiber (GF). We find one VPN, LC4, makes contact at a proximal GF location approximately 24 hours after puparium formation (hAPF). A second VPN, LPLC2, contacts GF dendrites at distal locations 24 hours later and this segregation is maintained across development. During this period, a third VPN, LPLC1, makes contacts with GF that aren't maintained into adulthood. To link timing of initial contacts to synapse formation, we label pre-synaptic active zones (brp-short) in LC4 while co-labeling endogenous DLG1, a post-synaptic scaffolding protein, in GF. Surprisingly, we observe brp-short/DLG1 appositions as early as 24hAPF. We compare these data with previously published single cell RNA-sequencing data generated for all three VPNs across development and find significant upregulation of select synaptic genes between 48-60hAPF compared to 24hAPF. These data suggest that although synaptic scaffolding proteins are present during initial contact, synaptic function is established later. To further determine when LC4 and GF are functionally coupled, we perform whole-cell patch clamp electrophysiology on the GF across pupal stages while activating LC4 via optogenetic stimulation in a novel *ex-plant* pupal preparation. Finally, we investigate how early LC4 loss, through overexpression of an inward-rectifying potassium channel Kir2.1, affects GF

connectivity and output. Loss of the majority of LC4 neurons results in a significant increase in GF/ LPLC2 glomerulus colocalization, suggesting the GF circuit compensates in the absence of a major pre-synaptic partner. Future work will investigate the functional consequences of this compensation. Our work establishes the GF escape circuit as an ideal sensorimotor developmental model to investigate complex wiring dynamics.

Disclosures: **B.W. McFarland:** None. **H. Jang:** None. **Y.Z. Kurmangaliyev:** None. **A. Nern:** None. **M.J. Parisi:** None. **K.C. Davis:** None. **T.J. Mosca:** None. **T.A. Godenschwege:** None. **C.R. von Reyn:** None.

Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.27

Topic: A.01. Neurogenesis and Gliogenesis

Support: HHMI

Title: Atoh1 drives the heterogeneity of the pontine nuclei neurons and promotes their differentiation

Authors: ***S.-R. WU**[1,2], J. C. BUTTS[1,2], J.-P. REVELLI[1,2], M. S. CAUDILL[1,2], H. Y. ZOGHBI[1,2,3];
[1]Baylor Col. of Med., Houston, TX; [2]Jan and Duncan Neurolog. Res. Inst., Houston, TX; [3]Howard Hughes Med. Inst., Houston, TX

Abstract: Pontine nuclei (PN) play an important role in motor function by mediating the communication between the cerebral cortex and the cerebellum. Previous studies in our lab showed that the PN are derived from *Atoh1*$^{+}$ progenitors and the mice heterozygous for an *Atoh1* null allele and a hypomorphic allele (*Atoh1*$^{S193A/-}$) exhibit migratory deficits and reduced size in the PN. It remains unclear, however, how Atoh1 regulates PN migration, and if the cellular identity of the PN neurons is altered when migration is compromised. In this study, we utilized an Atoh1-Cre knock-in mouse model in combination of a Cre-dependent TdTomato reporter to label *Atoh1*-lineage neurons and performed single-cell RNA-seq (scRNA-seq) on *Atoh1*-lineage neurons from the developing hindbrains of control and Atoh1$^{S193A/-}$ mice at embryonic stage 14.5 (E14.5). We demonstrated that scRNA-seq successfully captured the developing stages of the PN, including progenitor, intermediate progenitor, migrating and differentiated neurons. By quantifying the cellular proportion in different cell states, we observed an increase in progenitor cells and a decrease in migrating neurons in Atoh1$^{S193A/-}$ embryos, which suggests that Atoh1 is important for the cells to exit the progenitor stage. Moreover, by differential expressed gene analysis, we found that genes mediating migration were downregulated in Atoh1$^{S193A/-}$ embryos whereas Notch signaling, which is known for maintenance of progenitor pools, was upregulated. Importantly, we found that *Hrk* and *Bcl2l11* (known pro-apoptosis genes) were upregulated in

intermediate progenitors and migrating neurons of Atoh1$^{S193A/-}$ embryos, which may explain the reduced size in PN observed postnatally. We sought to perform scRNA-seq at later timepoint (E18.5). Interestingly, we found that the differentiated PN neurons at E18.5 can be categorized into four subtypes and one subtype identified by high *Prox1* expression was reduced in Atoh1$^{S193A/-}$ mice. These data suggest a potential role of Atoh1 in regulating the heterogeneity of the PN neurons. To exclude the possibility that the subtypes found at E18.5 were driven by maturity, we performed scRNA-seq at postnatal day 5 and uncovered six PN neuronal subtypes which were validated by RNA *in situ* hybridization. Importantly, one of the subtypes at P5 can also be identified by *Prox1* expression, confirming that there is a subtype-specific neuronal loss in Atoh1$^{S193A/-}$ mice. Together, this study sheds light on the role of Atoh1 in regulating the differentiation of PN neurons, reveals the heterogeneity of the PN neurons, and provides potential novel markers to study the function of different mature PN subtypes.

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Poster

607. Neurodevelopment: From Computer Models to Patients

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 607.28

Topic: H.12. Aging and Development

Support: VCU's CTSA (UL1TR002649 from the National Institutes of Health's National Center for Advancing Translational Science)
Wright Center Endowment Fund of the Virginia Commonwealth University

Title: Multisensory object permanence task in preterm children during early childhood

Authors: *V. CHU;
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Abstract: Children who are born preterm often experience developmental delays, including executive function challenges and cognitive delays (van Houdt et al., 2019). Research has shown that object permanence skills during toddlerhood is related to executive function abilities during early elementary school years in preterm children (Lowe et al., 2020). We explored object permanence in a multisensory assessment in 21 children born full term (aged 7-34 months) and 14 children born preterm (corrected age 6-33 months). The children completed two tasks. In task 1, children recovered a toy that was fully hidden by a visual barrier (blanket). This task is similar to stage 4 in Piaget's six stages of object permanence, typically achieved around 8-12 months in typically developing children (Piaget, 1954). In task 2, children recovered a sticker that is placed on their head, which is not visible to them. While this task does not involve tracking of hidden objects being moved through different hiding locations (stage 6, invisible displacement), it requires similar cognitive processing abilities, typically achieved between 18-24 months (Rast &

Meltzoff, 1995; Singer, 2018a). We examined two behavioral responses to the task: first, we recorded whether the children searched for the hidden sticker; and second, we recorded whether the children successfully recovered the sticker. Searching for the sticker demonstrates that the child has the working memory capability to represent the absent object without visual cue (object permanence). Successfully recovering the sticker demonstrates sensory processing abilities to feel spatially where the sticker is on their body and the motor skills to reach and peel the sticker. All children who participated in this study successfully completed task 1, demonstrating the presence of a lower level of object permanence abilities (stage 4). In task 2, all children under the age of 12 months were not successful in recovering the hidden sticker, however, a small percentage of children in both groups started reaching towards the direction of the hidden sticker in some trials. For the full term group, all the children searched for the hidden sticker 100% of the trials by 18 months, and were successful in recovering the hidden sticker 100% of the trials by 20 months. For the preterm group, children between 18-27 months were only occasionally searching for the hidden stickers. By 28 months, the children who were born preterm searched for and recovered the hidden stickers 100% of the time, 8 months after the full term group. Children who were born preterm showed delays in the development of higher-level object permanence skills, even after correcting for prematurity.

Disclosures: V. Chu: None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.01

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: FSIM:2019/R-Single/036

Title: The new mixed agonist of adenosine A_{2A}/A_{2B} receptors, MRS3997, affects CA1 hippocampal synaptic plasticity and myelination processes in OPC/DRG co-culture

Authors: *M. VENTURINI[1], F. CHERCHI[1], G. MAGNI[2], I. DETTORI[1], L. FRULLONI[1], C. SANTALMASI[1], K. A. JACOBSON[3], H. LEE[3], F. PEDATA[1], R. CORRADETTI[1], A. PUGLIESE[1], E. COPPI[1];
[1]Univ. of Florence, Florence, Italy; [2]Inst. of Applied Physics "Nello Carrara", Florence, Italy; [3]Natl. Inst. of Diabetes & Digestive & Kidney Dis., NIH, Bethesda, MD

Abstract: Adenosine is a neuromodulator acting in the peripheral and central nervous system by the activation of specific receptors: A_1, A_{2A}, A_{2B} and A_3. It is well established that, in the CA1 hippocampal region, the selective stimulation of the Gs-coupled A_{2A} and A_{2B} receptors (A_{2A}Rs, A_{2B}Rs) inhibits paired-pulse facilitation (PPF), an electrophysiological paradigm of short-term synaptic plasticity whose reduction reflects an increase in glutamate release. Of note, glutamate is also known as a chemoattractant for oligodendroglial precursor cells (OPCs) as it stimulates

their migration and differentiation into mature oligodendrocytes, thus facilitating myelination of active neurons. Myelination processes are also influenced by adenosine and we previously demonstrated that the selective activation of A_{2A}Rs, as well as A_{2B}Rs, reduces OPCs differentiation *in vitro* (Coppi et al., 2013; 2020). We hypothesized, therefore, that the synergic stimulation of these Gs-coupled adenosine receptors might be advantageous to different brain functions. By taking advantage of the recently synthesized compound MRS3997, a mixed A_{2A}R/A_{2B}R agonist (Gao et al., 2014), we explored the role of synergic A_{2A}R/A_{2B}R activation on PPF, by electrophysiological recordings in the CA1 region of rat hippocampal slices, and on myelin deposition in OPC/dorsal root ganglion (DRG) neuron co-cultures, by immunocytochemistry. We demonstrate that MRS3997 reduced PPF (P2/P1 ratio: from 1.83±0.07 in ctrl to 1.74±0.07 in 300 nM MRS3997, $P<0.05$). Similarly, the selective A_{2B}R agonist BAY60-6583 (200 nM), or the selective A_{2A}R agonist CGS21680 (50 nM), applied alone or together (BAY60-6583+CGS21680) reduced PPF thus, increasing glutamate release. Furthermore, when chronically (14 days) applied to DRG/OPC co-cultures, MRS3997 (200 nM) increased axonal myelination without affecting total myelin basic protein (MBP) expression, an effect shared by the selective A_{2B}R agonist BAY60-6583 (1 μM). Hence, we conclude that the newly synthetized compound MRS3997 produces effects consistent with the simultaneous activation of adenosine "A_2Rs": i.e. it increased glutamate release at hippocampal level and enhanced axonal myelination in DRG/OPC co-cultures, thus hypothesizing an advantage in the potential clinical translation of this molecule.

Disclosures: **M. Venturini:** None. **F. Cherchi:** None. **G. Magni:** None. **I. Dettori:** None. **L. Frulloni:** None. **C. Santalmasi:** None. **K.A. Jacobson:** None. **H. Lee:** None. **F. Pedata:** None. **R. Corradetti:** None. **A. Pugliese:** None. **E. Coppi:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.02

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: FISM: 2019/R-Single/036

Title: Oligodendrocyte Differentiation and Myelination are modulated via Adenosine "A2" Receptor Activation

Authors: *F. CHERCHI[1], M. VENTURINI[1], G. MAGNI[2], L. FRULLONI[1], C. SANTALMASI[1], F. ROSSI[2], E. COPPI[1], A. PUGLIESE[1];
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Abstract: Differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes (OLs) is a key event for axonal myelination in the brain; this process fails

during demyelinating pathologies, such as multiple sclerosis (MS). Adenosine is emerging as an important player in oligodendrogliogenesis, by activating all its metabotropic receptors: A1, A2A, A2B and A3 (A1R, A2AR, A2BR, A3R; Cherchi et al., 2021a). We demonstrated that Gs-coupled A2BRs and A2ARs decrease OPC differentiation by inhibiting tetraethylammonium (TEA)-sensitive potassium currents in primary OPC cultures (Coppi et al., 2013; 2020). Of note, 4-aminopyridine (4-AP), a broad-spectrum potassium channel blocker, was approved to improve motor skills in MS patients by promoting axonal conduction. In this study, the functional role of A2BRs and A2ARs was explored. By using patch-clamp recordings coupled to immunocytochemistry, we elucidated the role of "A2" receptors (A2Rs) on potassium currents in primary OPC cultures, on one hand, and on myelination processes in the same cells co-cultured with dorsal root ganglion (DRG) neurons, on the other hand. We first tested the effects of endogenous ligand adenosine (ADO, 0.1-50 μM), applied in the presence of selective A1R and A3R antagonists, on potassium currents in cultured OPCs. ADO decreases potassium currents similar to what has been observed in the presence of selective A2AR or A2BR agonists, CGS21680 and BAY60-6583, respectively. Chronic treatment (14 days) of OPC/DRG co-cultures with BAY60-6583 (1 μM) and/or CGS21680 (10 nM) increased myelin deposition without modifying total myelin basic protein (MBP) expression. Moreover, current-clamp experiments in isolated DRG neurons show that BAY60-6583 (1 μM) increased action potential firing, an effect prevented by the selective A2BR antagonist PSB-603 (10 nM). In conclusion, our data show that A2Rs activation inhibits potassium currents in OPC and increases myelin deposition along axons in OPC/DRG co-cultures, probably by enhancing action potential firing in DRG neurons, an important event involved in OPC migration and differentiation (Cherchi et al., 2021b). These results suggest that A2R activation modulates different functions in oligodendrogliogenesis, depending on their cellular localization, thus representing valuable targets in demyelinating pathologies, such as MS.

Disclosures: **F. Cherchi:** None. **M. Venturini:** None. **G. Magni:** None. **L. Frulloni:** None. **C. Santalmasi:** None. **F. Rossi:** None. **E. Coppi:** None. **A. Pugliese:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.03

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: K01MH123757
R01MH120212

Title: Evidence for conditional targeting of opioid receptors to primary cilia

Authors: *R. R. FAGAN[1], D. F. LEE[2], G. SCHERRER[2], M. VON ZASTROW[3], A. T. EHRLICH[1];
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Dept. of Pharmacol., The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Dept Psychiatry, UCSF, San Francisco, CA

Abstract: The primary cilium is a specialized microdomain present on virtually every cell type, including adult neurons. Cilia are a site of signaling for a select subset of G protein-coupled receptors (GPCRs), including neuromodulatory receptors. Considering that targeting of GPCRs to cilia is highly specific, it stands to reason that cilia-localized signaling by these receptors could have a profound impact on overall neuronal excitability and function. However, our present understanding of which neuromodulatory GPCRs are targeted to neuronal cilia, and which are excluded, remains incomplete. Here, we demonstrate that the Mu opioid receptor (MOR), a major target for opioid analgesics, is efficiently targeted to the primary cilium under certain conditions. Careful inspection of brain sections from MOR-Venus reporter mice revealed MOR localization to neuronal primary cilia in multiple brain regions. Interestingly, within the same region MOR-Venus ciliary localization varied, suggesting some conditional regulation of MOR trafficking. We further examined the mechanisms underpinning MOR cilia trafficking in the ciliated cell culture model, mouse inner medullary collecting duct (IMCD3) cells. We defined the structural determinants and identified critical trafficking components required for MOR cilia enrichment. Finally, we found that endogenous MOR localized to primary cilia in embryonic and adult medial habenula neurons. Taken together, these data provide evidence for conditional MOR localization to neuronal primary cilia under particular conditions but not others. Furthermore, these results suggest another potential facet of ciliary function in the CNS and of neuromodulation by opioids.

Disclosures: R.R. Fagan: None. **M. Von Zastrow:** None. **A.T. Ehrlich:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.04

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIDA R01DA035958

Title: Opioid mediated adaptations in the basolateral amygdala circuitry.

Authors: *J. P. RONSTRÖM[1], N. JOHNSON[2], S. JONES[2], H. A. WADSWORTH[1], J. BRUNDAGE[3], V. STOLP[2], N. M. GRAZIANE[4], Y. SILBERMAN[4], J. YORGASON[2]; [1]Brigham Young Univ., [2]Brigham Young Univ., Provo, UT; [3]Univ. of Utah, Salt Lake City, UT; [4]Pennsylvania State Univ., Hershey, PA

Abstract: Basolateral amygdala (BLA) activity is thought to initiate some aspects of anxiety, which is implicated drug use and withdrawal. BLA principle neurons are under feedforward inhibitory control from an adjacent cluster of intercalated GABAergic neurons referred to as lateral parascapsular (LPC) neurons. Mu and delta opioid receptors were observed in LPC

GABA neurons using confocal microscopy. Patch-clamp electrophysiology recordings were performed in LPC neurons in naive, saline and morphine treated GAD67-GFP transgenic mice. Changes in adenylyl cyclase circuit activity were examined. Selective opioid agonists inhibit LPC neurons, which is rescued by the application of the opioid antagonist naloxone. Morphine exposure and withdrawal in vivo resulted in changes in LPC physiology, resulting in decreased excitability. The adenylyl cyclase pathway becomes active during withdrawal in morphine treated mice. Specifically, phosphodiesterase inhibition, which increases cAMP levels, resulted in increased sIPSCs in LPC neurons of morphine, but not saline treated mice. Additionally, activation of the inhibitory adenosine A1A receptor (A1AR) resulted in sIPSC decreases that were reversed to a greater extent with A1AR antagonists in morphine and saline treated mice. Together, these data suggests a secondary adaptation in LPC circuit function that results in greater inhibition of LPC neurons. Lastly, behavioral experiments were conducted - the marble burying assay and the open field locomotion test. Double floxed mu-opioid receptor transgenic mice received intra BLA AAV-cre-GFP or AAV-GFP infusions to reduce MOR expression. Mice were then treated with morphine or saline (IP) for 1 week. Morphine mice had increased anxiety-like behavior in marble burying task, which was occluded in mu-opioid receptor knockdown mice. Together, these data highlight the importance of the mu-opioid receptor in BLA paracapsular neurons in morphine induced circuit adaptations and anxiety-like behavior.

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Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.05

Topic: E.03. Basal Ganglia

Support: NIH Grant AA025932
NIH Grant AA021505

Title: Striatal mu-opioid receptor activation triggers direct-pathway GABAergic plasticity and induces negative affect

Authors: J. WANG[1], W. WANG[1], X. XIE[1], X. ZHUANG[1], Y. HUANG[1], T. TAN[1], H. GANGAL[1], Z. HUANG[1], W. PURVINES[1], X. WANG[1], A. STEFANOV[1], R. CHEN[1], E. YU[1], M. HOOK[1], Y. HUANG[2], E. DARCQ[3];
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Abstract: Withdrawal from chronic opioid use often causes hypodopaminergic states and negative affect, which drives relapse. Direct-pathway medium spiny neurons (dMSNs) in the

striatal patch compartment contain high levels of mu-opioid receptors (MORs). It remains unclear how chronic opioid exposure affects these MOR-expressing dMSNs and their striatopallidal and striatonigral outputs to induce negative emotions and relapse. Here, we report that MOR activation acutely suppressed GABAergic striatopallidal transmission in habenula-projecting globus pallidus neurons. Notably, repeated administrations of a MOR agonist (morphine or fentanyl) potentiated this GABAergic transmission. We also discovered that intravenous self-administration of fentanyl enhanced GABAergic striatonigral transmission and reduced the firing activity of midbrain dopaminergic neurons. Importantly, fentanyl withdrawal caused depression-like behaviors and promoted the reinstatement of fentanyl-seeking behaviors. These data suggest that chronic opioid use triggers GABAergic striatopallidal and striatonigral plasticity to induce a hypodopaminergic state, promoting negative emotions and leading to relapse.

Disclosures: J. Wang: None. **W. Wang:** None. **X. Xie:** None. **X. Zhuang:** None. **Y. Huang:** None. **T. Tan:** None. **H. Gangal:** None. **Z. Huang:** None. **W. Purvines:** None. **X. Wang:** None. **A. Stefanov:** None. **R. Chen:** None. **E. Yu:** None. **M. Hook:** None. **Y. Huang:** None. **E. Darcq:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.06

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Cannabidiol-derived variants as potential negative allosteric modulators at the mu opioid receptor

Authors: *T. BOSQUEZ, J. GUDORF, M. VANNIEUWENHZE, A. STRAIKER;
Indiana University-Bloomington, Indiana University-Bloomington, Bloomington, IN

Abstract: The US has experienced one of the most severe waves of the opioid epidemic during the last decade. The drugs with the highest abuse potential are synthetic opioids, such as fentanyl. The increased potency of these synthetic opioids can reduce the therapeutic effect of Narcan (naloxone), which competitively targets the primary (orthosteric) binding site of the mu opioid receptor (μOR). As a result, these potent drugs are a leading cause of many recent opioid overdose deaths. In addition to an orthosteric site, receptors like the μOR, also contain a distinct secondary binding site called an allosteric site. Ligands that occupy this site are called allosteric modulators, as they modulate the structure of the receptor and change the binding affinity of the orthosteric site. Ligands that reduce receptor activity are called negative allosteric modulators (NAMs). NAMs have the potential to reduce synthetic opioid induced signaling without competing for the orthosteric site. Cannabidiol (CBD), one of the main constituents of *Cannabis*, has been implicated as a NAM at the μOR; however, with a relatively low affinity.
After an initial screen of ~50 CBD (JGCx) analogs, we tested seven promising analogs to

determine if these had an improved affinity and more potent NAM activity at μOR, using an *in-vitro* cAMP-based assay involving a fluorescent protein to measure cAMP accumulation in μOR-HEK293 cells. When μ is activated, its coupling to the Gai/o G protein subunit leads to an inhibition of cAMP accumulation. Each CBD analog was tested against μOR agonists, DAMGO and fentanyl, to measure cAMP accumulation in our cells. Area under the curve analysis enabled comparing the signaling characteristics of CBD and its 7 analogs.
CBD and its analogs all interfered with DAMGO- and fentanyl-induced μOR singling, by increasing cAMP production following their application to v μOR-HEK293 cells. Further analysis revealed the analogs were more potent than CBD (CBD IC50: 1.8μM; JGC2: 90nM; JGC13: 137nM; JGC21: 499nM; JGC22: 556nM; JGC25: 556nM; JGC26: 173nM; JGC31: 242nM) in interfering with fentanyl-induced signaling.
These results indicate that CBD, but also 7 CBD analogs, have the potential to be considered NAMs, as they attenuate μOR activation in the presence of potent agonists. These results also suggest specific modifications to the CBD chemical structure make it possible to enhance the potency of the root compound. Understanding the role these structural modifications play in allosteric interactions will be important for the elucidation of the allosteric site at the μOR and will pave the way for developing a novel class of potentially therapeutic compounds that curb opioid signaling.

Disclosures: **T. Bosquez:** None. **J. Gudorf:** None. **M. VanNieuwenhze:** None. **A. Straiker:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.07

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: PJT-162103

Title: Mitochondrial localization, import pathway and function of the delta-opioid receptor

Authors: *L. CÔTÉ[1], J. DEGRANDMAISON[1], S. GÉNIER[2], E. GAUTHIER[2], I. BÉRUBÉ[1], P. LABRECQUE[2], S. LABBÉ[3], L. GENDRON[1], J.-L. PARENT[2];
[1]Pharmacologie-Physiologie, [2]Médecine, [3]Biochimie et de Génomique Fonctionnelle, Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: GPCRs were classically thought to signal from the plasma membrane but were recently shown to be functional in membrane-bound organelles such as mitochondria. However, how GPCRs are targeted to mitochondria and their mitochondrial interactomes are still unknown. Interestingly, many studies show the mito and neuroprotective roles of the delta-opioid receptor (DOPr), a GPCR known to be mainly intracellular and barely expressed to the cell surface. Here, studies performed by LC-MS/MS analyses on immunoprecipitated FLAG-tagged DOPr from

brain homogenates of knock-in mice revealed numerous endogenous mitochondrial proteins as DOPr interactors in its native environment. Confocal and electron microscopy, as well as cellular fractionation assays indicated that DOPr is localized at the mitochondrial outer membrane. Since several members of the translocase of the outer membrane (TOM) complex were identified in our LC-MS/MS analyses, we further investigated the potential contribution of this family of proteins in DOPr mitochondrial import pathway. We also identified many components of the respiratory chain and the core proteins involved in mitochondrial dynamics. We first confirmed the interaction between DOPr and Tom20, Tom22 and Tom70 by co-immunoprecipitations, and reported that Tom70, the mitochondrial import receptor, could directly bind multiple DOPr domains. In vivo, knockout of Tom70/Tom71 in yeast cells decreased DOPr mitochondrial targeting. Interestingly, residues 50 to 100 of DOPr (DOR50) fused to eGFP caused robust targeting of the fluorescent protein to mitochondria, while the corresponding sequence of MOPr (MOR71) failed to do so. Mutagenesis analyses revealed that a single glycine residue embedded within DOR50 acts as a major molecular determinant for its mitochondrial targeting. Respiration experiments carried out on isolated mitochondria also showed that DOPr reduces the oxygen consumption rate. Confocal microscopy analyses suggest that treatment of DOPr expressing cells with selective DOP agonists regulates mitochondrial morphology and is protective against mitochondrial injury. We report the mitochondrial interactome, function and import mechanism of a GPCR, namely the DOPr.

Disclosures: L. Côté: None. **J. Degrandmaison:** None. **S. Génier:** None. **E. Gauthier:** None. **I. Bérubé:** None. **P. Labrecque:** None. **S. Labbé:** None. **L. Gendron:** None. **J. Parent:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.08

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIDA DA044999

Title: Jwh-133 (cannabinoid receptor 2 agonist) chronic administration increases ectopic ovarian tumor growth and endocannabinoids (anandamide and 2-arachidonoyl glycerol) levels in immunocompromised scid female mice

Authors: *I. CASTRO-PIEDRAS[1], M. MCHANN[2], J.-L. REDONDO[1], J. GUINDON[3]; [2]Pharmacol. and Neurosci., [3]Dept. of Pharmacol. and Neurosci., [1]Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Cannabinoid-based therapies are increasingly being used by cancer patients to treat chemotherapy-induced nausea and vomiting. Recently, cannabinoids have gained increased attention for their effects on cancer growth. Indeed, the effect of CB_2 (JWH-015, JWH-133)

agonists on breast cancer models have shown to reduce the size of breast cancer tumors. However, these studies assessing breast cancer progression were using CB_2 agonist administered early into the cancer progression. Therefore, assessing their effects on already established tumors is a critical need. In our study, we evaluate tumor growth using an ectopic xenograft ovarian (SKOV-3 and OVCAR-5) cancer model. The impact of chronic (30 days) administration of CB_2 (JWH-133) agonist will be evaluated and started on 30 days of ectopic ovarian tumors. We will then evaluate and determine the mechanisms involved in ovarian cancer tumor growth by measuring levels of anandamide and 2-arachidonoyl glycerol as well as protein levels of CB_1, CB_2, ERα, ERβ, GPER, TNFα, IL-1β and IL-6 in ovarian and tumor tissues. Our results demonstrate a significant increase in ectopic ovarian tumor growth following chronic administration of JWH-133, but not after administration of cannabinoid receptor 1 agonist. Ovarian cancer tumor tissues chronically (30 days) treated with JWH-133 in comparison to vehicle treated groups showed an increase in endocannabinoid (AEA and 2-AG) and protein (CB_2 and TNFα) levels with a decrease in GPER protein levels. Interestingly, our study emphasizes the importance of studying the impact of cannabinoid compounds on already established tumors to improve our understanding of cannabinoid-based therapies and, therefore better address clinical needs in cancer patients.

Disclosures: I. Castro-Piedras: None. **M. McHann:** None. **J. Redondo:** None. **J. Guindon:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.09

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIDA DA044999

Title: Dose and sex-dependent effects of jnk signaling on tolerance to cp55,940 in cb1 desensitization-resistant mutant mice

Authors: M. MCHANN[1], I. CASTRO-PIEDRAS[2], D. J. MORGAN[3], ***J. GUINDON**[1]; [2]Pharmacol. and Neurosci., [1]Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [3]Dept. of Biomed. Sci., Marshall Univ., Huntington, WV

Abstract: Introduction: Studies from our group and others have found sex-differences in the response to cannabinoid compounds (Blanton et al., 2021). Chemotherapy-induced peripheral neuropathy (CIPN) is a clinical challenge for cancer patients. The development of novel targeted therapies with long-term efficacy in alleviating CIPN is an ongoing focus of preclinical research. However, our current understanding of the mechanisms underlying tolerance to cannabinoid compounds remains elusive as does the contribution of sex differences to this process. **Methods**: The objective of our current work is to assess in S426A/S430A female and male mutant mice

whether JNK (SU 3327) inhibitor, CP55,940 (mixed CB_1/CB_2 receptor agonist) alone or in combination demonstrate sex-specific antinociceptive effects and delay in the development of tolerance using cisplatin (5 mg/kg/week) as a CIPN model. We also evaluated co-immunoprecipitation of JNK1 and JNK2 with β-arrestin 2 in HEK293-CB_1 cells. **Results**: Our study found that SU 3327 (3 mg/kg i.p.) partially reversed mechanical (von Frey) and cold (acetone) allodynia in male and female KI mice from day 8 to day 35. However, this effect was not observed at a lower dose of SU 3327 (1 mg/kg i.p.). When low dose SU 3327 (1 mg/kg i.p.) was combined with CP55,940 (0.3 mg/kg i.p.), there was a delay in the development of tolerance to the effects of CP55,940 on mechanical and cold allodynia in female mice. Indeed, tolerance to the effects of CP 55,940 on mechanical allodynia developed on day 28 for CP55,940 alone and on day 33 for SU 3327 + CP55,940 for female KI mice. Tolerance to effects of CP55,940 on cold allodynia developed on day 27 for CP55,940 alone and on day 32 for SU 3327 + CP55,940 for female KI mice. For male KI mice, tolerance to the effects of CP55,940 on mechanical and cold allodynia developed on day 33 and on day 32 for CP55,940 alone or combined with SU 3327, respectively. To better understand a possible mechanism for these findings we performed co-immunoprecipitation experiments and found that JNK2 and β-arrestin 2 form a complex in CB_1-espressing HEK293 cells. **Conclusions**: Our results illustrate the important role of sex in the development of cannabinoid tolerance in the context of chronic pain and the contribution of sex-specific mechanisms of action

Disclosures: M. McHann: None. **I. Castro-Piedras:** None. **D.J. Morgan:** None. **J. Guindon:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.10

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH DA044999

Title: Mutant mice expressing an internalization-resistant form of CB_1R display enhanced cannabinoid tolerance.

Authors: M. PISCURA[1], **A. HENDERSON-REDMOND**[1], M. MAULIK[1], K. DESCHEPPER[1], C. LULEK[1], J. GUINDON[2], *D. J. MORGAN[1];
[1]Marshall Univ., Marshall Univ., Huntington, WV; [2]Texas Tech. Univ. Hlth. Sci. Ctr., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Although cannabinoids such as delta-9-tetrahydrocannabinol (Δ9-THC) exhibit clinical efficacy in pain, tolerance to the antinociceptive effects develops with repeated treatment. The focus of our work is to investigate the mechanisms responsible for the acute response and tolerance to different cannabinoid agonists. We previously found that tolerance to

cannabinoids is reduced in S426A/S430A mutant mice expressing a desensitization-resistant form of cannabinoid receptor 1 (CB1R) that disrupts the classic mechanism of G protein-coupled receptor kinase (GRK)/βarrestin2-mediated CB1R desensitization [1]. The objective of our current work is to assess the role of CB1R internalization and trafficking on cannabinoid tolerance. This objective will be achieved using a novel six point mutant mouse strain expressing an internalization-resistant form of CB1R that was recently produced in our laboratory.Knock-in mice were produced that express serine/threonine to alanine point mutations for six putative G protein-coupled receptor kinase (GRK) phosphorylation sites in the distal C-terminus of CB1R that are required for the efficient internalization of the receptor in transfected cells. The acute response to CP55,940 was assessed by performing cumulative dose response curves. Antinociception was measured using the tail-flick and hotplate tests while cannabinoid-induced hypothermia was assessed by measuring core body temperature. Tolerance to the antinociceptive and hypothermic effects of once daily injections of 0.6 mg/kg CP55,940 was determined. We find that the maximal acute effect for CP55,940 on hypothermia and tail-flick antinociception is reduced in six point mutant mice relative to wild-type littermate controls. We also find a shorter duration for the acute hypothermic effects of CP55,940 in six point mutant mice. Previous work has demonstrated that βarrestin2-mediated desensitization of CB1R modulates the magnitude and duration of acute responses for cannabinoids. Six point mutant mice also display enhanced tolerance to the antinociceptive and hypothermic effects of 0.6 mg/kg CP55,940 relative to wild-type littermate controls. This work establishes six point mutant mice as a novel model to study the role of CB1R internalization, trafficking, and resensitization in vivo. Preliminary data shows that cannabinoid tolerance is increased in six point mutant suggesting that the normal processes of internalization, traffickly, and resensitization of CB1R might play an important role in counteracting the development of cannabinoid tolerance.

Disclosures: **M. Piscura:** None. **A. Henderson-Redmond:** None. **M. Maulik:** None. **K. DeSchepper:** None. **C. Lulek:** None. **J. Guindon:** None. **D.J. Morgan:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.11

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant NS112194

Title: Activity of protein kinase C controls the reduced efficacy of cannabinoid receptor type 1 in medial prefrontal cortex after neuropathic pain

Authors: D. CHAO[1], A. LI[1], H. TRAN[1], L. KRAUSE[2], Q. H. HOGAN[1], C. J. HILLARD[3], ***B. PAN**[1];
[1]Anesthesiol., [2]Med. Col. of Wisconsin, Milwaukee, WI; [3]Med. Col. Wisconsin, Milwaukee, WI

Abstract: Many patients with chronic pain conditions suffer from depression. In our previous report, we found that afferent noxious inputs after painful nerve injury compromise activity-dependent endocannabinoid (eCB) signaling in the medial prefrontal cortex (mPFC), resulting in depression. Protein kinase C (PKC) and cAMP/protein kinase A (PKA) pathways can regulate eCB receptor type-1 (CB1R)-mediated synaptic transmission. In this study, we explored the role of PKC and PKA in reduced eCB signaling after neuropathic pain (spared nerve injury, SNI). Four weeks after SNI, rats with SNI developed hypersensitivity to punctate mechanical stimulation to plantar skin of hind paw, and depression. Brain tissues containing mPFC were harvested for radioligand-based CB1R binding assay and brain slice electrophysiological recordings. With the radioligand-based CB1R binding assay, no different CB1R binding ability was found after SNI in both male and female rats. Effects of PKA agonist, forskolin, and blocker, H-89, on evoked inhibitory postsynaptic currents (eIPSCs) were not changed after SNI. PKC blocker, chelerythrine (CHEL), fully blocked effects of CB1R agonist, WIN-55212-2, on eIPSC. Activating PKC with phorbol 12-myristate 13-acetate reversed the reduced effects of WIN-55212-2 on eIPSC and miniature IPSC after SNI. These data suggest that reduced PKC activity controls the efficacy of CB1Rs in mPFC after neuropathic pain, and targeting PKC signaling might be a potential strategy to treat depression after neuropathic pain.

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Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.12

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: William Townsend Porter Pre-doctoral Fellowship from the American Physiological Society

Title: Increased cannabinoid 1 receptor activity following reduced uterine perfusion pressure dampens pentylenetetrazol-induced seizures at gestational day 18.5 in mice

Authors: *M. JONES-MUHAMMAD[1], T. PRYOR[2], Q. SHAO[2], K. FREEMAN[3], J. P. WARRINGTON[2];
[2]Neurol., [3]Psychiatry, [1]Univ. Of Mississippi Med. Ctr. Grad. Program In Neurosci., Jackson, MS

Abstract: Preeclampsia, a hypertensive disorder of pregnancy, can advance to eclampsia, if new onset seizures occur. While increased sensitivity to pentylenetetrazol (PTZ)-induced seizures was reported in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia, the underlying mechanisms are not clear. We hypothesized that the neuromodulatory role of the endocannabinoid system is impaired in RUPP mice, thus increasing RUPP-induced seizure sensitivity. Pregnant mice underwent sham (n=5) or RUPP (n=5) surgery on gestational day

(GD) 13.5. On GD 18.5, the hippocampus was harvested for Western blot to analyze the expression of the endocannabinoid system receptor, cannabinoid receptor 1(CB1R), and enzymes, N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD), Diacylglycerol lipase (DAGL), and monoacylglycerol lipase (MAGL). To determine if blocking the CB1R changes sensitivity to PTZ-induced seizures, another set of sham (n=7) and RUPP (n=7) mice were pretreated with 10 mg/kg of rimonabant, video monitored for 15 minutes, injected with 40 mg/kg of PTZ and video monitored for an additional 30 minutes. Mice were sacrificed immediately after seizure monitoring. Seizures were scored using a modified 7-point Racine scale. Western blot data was analyzed using the Mann-Whitney test and seizure behavior was analyzed using Mixed-effects analysis. In RUPP mice, hippocampal CB1R ($p=0.056$) and NAPE-PLD increased ($p=0.056$), with no change in DAGL ($p=0.452$), and a significant reduction in MAGL expression ($p=0.032$). Rimonabant induced a maximum seizure score of 4 in both groups; however, PTZ injection after rimonabant pretreatment increased seizure scores in sham ($p<0.001$) and non-significantly increased scores in RUPP mice ($p=0.075$). RUPP mice also had lower seizure scores than sham mice ($p=0.019$) post-PTZ. Moreover, sham mice had reduced latency to seizures after PTZ ($p=0.019$) while there was no further decrease in seizure latency in RUPP after PTZ ($p=0.374$). Together, these results suggest that RUPP increases endocannabinoid system activity (significant changes in the expression of enzymes important for CB1R ligand synthesis and degradation), suggesting abnormal neuromodulatory activity at baseline. Furthermore, a reduced response to PTZ-induced seizures in RUPP mice after blocking CB1R, suggests that baseline activity of the CB1R is dysregulated following the RUPP procedure. Ongoing work will investigate whether RUPP mice have abnormal concentration of the ligands, anandamide and 2-arachidonylglycerol, determine where in the hippocampus CB1R increases, and explore the effects of CB1R agonists on seizure activity following RUPP.

Disclosures: M. Jones-Muhammad: None. **T. Pryor:** None. **Q. Shao:** None. **K. Freeman:** None. **J.P. Warrington:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.13

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Midwestern University Intramural Research Funding
Midwestern University One Health Initiative Funding

Title: Synthetic cannabinoid receptor agonists CP-55,940 and WIN 55,212-2 inhibit spontaneous calcium oscillations via the cannabinoid 1 (CB1) receptor in a dose-dependent manner in murine primary cerebrocortical neuron cultures

Authors: A. BASITH[1], J. ABURAS[2], ***M. PIERCE**[2];
[1]Biomed. Sci., [2]Pharmacol., Midwestern Univ., Downers Grove, IL

Abstract: BACKGROUND
Preliminary studies have suggested that cannabinoid agonists may be helpful in treating a number of neurological disorders including epilepsy and chronic pain, as well as psychopathologies including anxiety and post-traumatic stress disorder. Endocannabinoids and their receptors are highly expressed during cortical development, and play an important role in neuronal proliferation and plasticity. Cannabinoid 1 receptors (CB1) are G-protein coupled receptors that signal through Gi/o and Gs and are expressed in cortical neurons, among other cell populations. We hypothesize that Gi-mediated potassium currents stimulated by synthetic cannabinoid agonists will result in inhibition of spontaneous calcium oscillations in murine primary cortical neurons.
METHODOLOGY
Functional assays using Fluo8-AM were performed on the FLIPR2 to determine effects of the cannabinoid agonists on spontaneous calcium oscillations in murine primary cerebrocortical neuron cultures at days *in-vitro* (DIV) 12. Synthetic cannabinoid receptor agonists CP 55,940 is a CB1 and CB2 receptor agonist and WIN 55,212-2 is a CB1 receptor agonist. AM281 is a CB1 receptor antagonist. Graphpad Prism 9.3.1 software was used to analyze time and concentration response relationships. Calcium oscillation data is a plot of the raw Fluo8 fluorescence reads over time.
RESULTS
Cannabinoid receptor agonists CP-55,940 and WIN 55,212-2 abrogated spontaneous calcium oscillations at 10 and 1 micromolar doses. These effects were rescued with 10 minutes of pretreatment with CB1-receptor antagonist AM-281, suggesting that CP 55,940 and WIN 55,212-2 mediated effects on spontaneous calcium oscillations are occurring through the CB1 receptor. CONCLUSIONS
Collectively, these data will provide key findings into how cannabinoid agonists affect neuronal signaling, so that these effects can better inform cannabinoid-mediated therapeutic development in humans.

Disclosures: A. Basith: None. **J. Aburas:** None. **M. Pierce:** None.

Poster

608. Adenosine, Opioid, and Endocannabinoid Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 608.14

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH grant R00AA027806
University of Pittsburgh start-up funds

Title: Sex differences in metabotropic glutamate $mGlu_1$ and $mGlu_5$ regulation of prefrontal cortex parvalbumin interneurons and responses to ethanol

Authors: C. B. FABIAN[1], L. G. CARLEY[1], A. S. FERRANTI[2], S. M. THOMPSON[1], *M. E. JOFFE[1];
[1]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [2]Vanderbilt Univ., Nashville, TN

Abstract: Proper function of the prefrontal cortex (PFC) is essential for the top-down regulation of motivated and affective behaviors. Population-level differences in PFC function have been related to the diagnosis of several neuropsychiatric diseases, including schizophrenia, major depressive disorder, and alcohol use disorders (AUD). Each of these conditions presents with pronounced differences between sexes: women with schizophrenia develop the disease later than men, women exhibit higher prevalence of major depressive disorder, and women who develop AUD proceed through disease milestones more rapidly than male counterparts. As such, there is great motivation to identify cellular and synaptic signaling mechanisms that guide sex differences in PFC function. Parvalbumin-expressing inhibitory neurons (PV-INs) are a key subpopulation of GABAergic cells within PFC. Previous mouse studies from our lab have found that PFC PV-INs display basal sex differences in membrane physiology parameters and undergo sex-dependent adaptations following chronic drinking; however, the cellular elements and synaptic plasticity mechanisms that mediate these sex-dependent effects remain unclear. Here, we investigate Group 1 metabotropic glutamate receptors ($mGlu_1$ and $mGlu_5$), two related G protein-coupled receptors that are highly expressed in PFC PV-INs. Using transgenic mice expressing tdTomato fluorescent protein in PV-INs and a combination of selective pharmacological tools, we made whole-cell patch clamp recordings of layer 5 prelimbic PV-INs to examine basal sex differences in $mGlu_1$ and $mGlu_5$ modulation of PV-IN membrane and synaptic physiology. We also assessed sex differences in cell type-specific receptor expression at the transcript level via RNAscope. Finally, mice with genetic deletion of $mGlu_5$ receptors from PV-INs were used to examine unconditioned responses and behavioral adaptations to ethanol. While ethanol reward learning was not different in PV-$mGlu_5^{-/-}$ mice of either sex, female but not male PV-$mGlu_5^{-/-}$ mice exhibited decreased ethanol-induced hyperactivity following ethanol administration. Taken together, these studies identify $mGlu_1$ and $mGlu_5$ receptors as candidate signaling molecules involved in sex differences in PV-IN activity, PFC function, and behaviors relevant for AUD and other psychiatric diseases.

Disclosures: C.B. Fabian: None. **L.G. Carley:** None. **A.S. Ferranti:** None. **S.M. Thompson:** None. **M.E. Joffe:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.01

Topic: B.05. Synaptic Plasticity

Support: CIHR Grant 142420
HSF GIA G-15-0009399

Title: Time-dependent remodeling of the habenulo-raphe pathway in an animal model of post-stroke depression

Authors: *S. MAILLÉ, S. D. GEDDES, D. LEMELIN, S. ASSADZADA, J.-C. BEIQUE; Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Stroke is a leading cause of death and the primary cause of adult long-term disability in the developed world. Despite increasingly efficient rehabilitation programs, stroke survivors experience an elevated incidence of symptoms of major depression which, beyond the emotional suffering, also undermine recovery outcomes by reducing the motivation of patients to adhere to a sufficiently intense rehabilitation program. Human and animal studies have linked the incidence of post-stroke depression to the extent of damage to the prefrontal cortex (PFC). Here, we hypothesized that PFC stroke promotes the development of depressive phenotypes by triggering maladaptive network remodeling in downstream, mood-related networks. The PFC and the epithalamic lateral habenula (LHb) are limbic structures that send powerful, top-down axonal projections to the serotonergic dorsal raphe nucleus (DRN), a key neuronal hub for mood regulation. We used viral, optogenetic and electrophysiological strategies to outline the functional architecture of the PFC and LHb projections to DRN. We found that an endothelin-1-mediated stroke in PFC triggered a time-dependent remodeling of key functional features of the glutamatergic input from the LHb to DRN 5-HT neurons, in a way that resembled homeostatic synaptic scaling following long-term network silencing. At LHb-DRN synapses, this was associated with synaptic insertion of calcium permeable, GluA2-lacking AMPARs and an increase in the relative contribution of AMPARs to optogenetically-evoked EPSCs. Furthermore, using a strontium-based quantal analysis approach we found that this time-dependent remodeling resulted in increased quantal size and sharpened decay kinetics at individual LHb-DRN synapses. Because the LHb-DRN pathway is believed to encode negative valence emotional features such as aversion and anticipation of threat, a maladaptive, homeostatic-like upregulation of this pathway may contribute to depressive symptomology following stroke.

Disclosures: S. Maillé: None. **S.D. Geddes:** None. **D. Lemelin:** None. **S. Assadzada:** None. **J. Beique:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.02

Topic: B.05. Synaptic Plasticity

Support: NIH R35 NS111562

Title: Coordination of homeostatic plasticity mechanisms in the developing mouse primary visual cortex

Authors: *W. WEN[1], G. TURRIGIANO[2];
[1]Biol., [2]Brandeis Univ., Brandeis Univ., Waltham, MA

Abstract: Neural circuits need to maintain overall stability in the face of ongoing perturbations, to ensure the fidelity of information transfer and storage during learning and development. This is achieved by a repertoire of homeostatic plasticity mechanisms that regulate the excitation, inhibition, or intrinsic excitability of neurons within the circuit. Two major forms of homeostatic plasticity, synaptic scaling (SS) and intrinsic homeostatic plasticity (IHP), have been observed in parallel under various experimental conditions and activity manipulation paradigms, raising the question of whether their induction is interdependent. Here, we used inhibitory DREADDs to chronically suppress activity in mouse primary visual cortex (V1) for 24h during the classic rodent visual system critical period (CP), a time where both SS and IHP have been observed in DREADD-positive pyramidal neurons (PN). Next, we interfered with molecular pathways that are known to be necessary for either the induction or the expression of SS, and measured the intrinsic excitability of affected PNs to probe whether IHP would still be intact under SS blockade. We first blocked SS by co-expressing DREADD and GluA2 c-terminal fragments (c-tail) in V1 PNs, and found that IHP was also eliminated in these neurons. We next abolished SS via a mechanistically distinct pathway, by scavenging the extracellular tumor necrosis factor alpha (TNFα), and also observed the absence of IHP. These results suggest that SS possibly initiates IHP through an unknown downstream signaling pathway. One candidate for this role is signaling through the NMDA receptor (NMDAR), which is unnecessary for SS expression, yet whose involvement in IHP is less studied. Therefore, we administered an NMDAR antagonist, CPP, to the animals in parallel with the DREADD paradigm, and found that IHP was occluded by NMDAR inactivation. These data suggest that signaling through NMDAR is likely involved in IHP induction and/or expression, and could potentially explain the crosstalk between SS and IHP. We plan to further investigate the role of NMDAR in IHP in cultured neocortical neurons, where we can take advantage of well-established molecular manipulations to induce both SS and IHP.

Disclosures: W. Wen: None. **G. Turrigiano:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.03

Topic: B.05. Synaptic Plasticity

Support: 5F31EY028431-02

Title: Bidirectional changes in pyramidal neuron excitability during dark exposure and light reintroduction are necessary for the reactivation of ocular dominance plasticity

Authors: *A. BORRELL[1], E. M. QUINLAN[2];
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Abstract: Monocular amblyopia is a common form of blindness induced by visual impairment to one eye during a "critical period" (CP) of early development when conditions in the visual cortex are highly permissive for synaptic plasticity. Following the CP, synapses become less plastic, and amblyopia becomes increasingly refractory to treatment. However, sensory deprivation via dark exposure (DE) lowers the threshold for Hebbian plasticity at cortico-cortical synapses resulting in an increase in the excitability of regular spiking (RS) pyramidal neurons in the visual cortex, (Bridi et al., 2018) and lowers the threshold for the activation of peri-synaptic proteolytic enzymes at thalamocortical synapses (Murase et al., 2019). Here we show that light reintroduction (LRx) following DE drives the excitability of RS single units (presumptive pyramidal cells) down from a homeostatic ceiling. To ask if this decrease in excitability during LRx is necessary for the reactivation of plasticity, we prevented this decrease with chemogenetic disinhibition of the cortex. Viral-mediated expression of inhibitory Gi-DREADD receptors (AAV-hsyn-FLEX-hM4D-mcherry) was employed in the binocular primary visual cortex (V1b) and in adult (P100+) PV-cre mice. Chronically implanted electrode arrays were used to simultaneously record single unit (SU) spiking output and visual-evoked local field potentials (VEPs) in awake head-fixed mice. Chemogenetic disinhibition of V1b significantly increased VEP amplitudes (21±15%; $n = 12$ mice; $p < 0.01$) and increased evoked and spontaneous SU spike rates across all layers (46±15% and 28±16%, respectively; $n = 11$ mice; $p < 0.05$) relative to uninfected PV-cre control mice. As expected, monocular deprivation (MD) following DE induced a significant negative shift in the ocular dominance indices of PV-cre control mice (-0.18±5; $n = 8$ mice; $p < 0.02$) across all cortical layers. However, no change in ocular dominance indices was observed in Gi-DREADD-infected mice, indicating that disinhibition of V1b during LRx blocks the reactivation of OD plasticity. Chemogenetic disinhibition of V1b did not prevent MD-induced decreases in physiologically derived monocular visual acuity of the deprived eye, consistent with previous reports that acuity and OD plasticity are mediated by changes in different anatomical loci (Stephany et al., 2014). Thus the decrease in neuronal excitability in response to LRx is critical for the reactivation of ODP plasticity in post-critical period mice.

Disclosures: A. Borrell: None. **E.M. Quinlan:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.04

Topic: B.05. Synaptic Plasticity

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Title: Division of labor among histone H3 lysine 4 methyltransferases regulates distinct facets of homeostatic synaptic plasticity

Authors: *T. TSUKAHARA[1,2,3], S. KETHIREDDY[3], K. BONEFAS[4], A. CHEN[4], B. L. SUTTON[3], K. GE[5], Y. DOU[6], S. IWASE[1,3], M. A. SUTTON[2,3];
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Abstract: Methylation at lysine 4 of Histone 3 (H3K4me) is an evolutionarily conserved post-translational modification that is associated with transcriptionally active areas in the genome. In mammals, the majority of H3K4me is placed by six writer enzymes, namely KMT2A-D, -F and -G (KMT2s). Interestingly, all six KMT2 genes are monogenetically associated with neurodevelopmental disorders, such as autism and intellectual disability, implying a non-redundant role for these H3K4me enzymes in the development and function of the central nervous system. The roles of KMT2s have been intensely studied in embryonic development, but the roles of these enzymes in postmitotic neurons remain largely unknown. Using genetics, electrophysiology, neuronal imaging, and gene expression profiling, we have found that all six H3K4me writer enzymes are expressed in excitatory neurons and engaged in an enduring form of synaptic plasticity to homeostatically maintain the stability of neuronal activity. Intriguingly, rather than playing overlapping roles, each KMT2 writer appears to play a specific and unique role in distinct facets of homeostatic synaptic plasticity (HSP). Significantly, introduction of H3K4 mutant and catalytic-dead models of KMT2s exhibit deficits in HSP, underscoring the critical roles of H3K4-methyltransferase function in addition to the other non-enzymatic and non-histone functions of the KMT2s. Taken together, our results indicate that the homeostasis of neural circuits is established via precise regulation of H3K4me via KMT2s, and disruption of homeostatic signaling might play a role in the pathogenesis of neurological disorders caused by mutations in KMT2s.

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Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.05

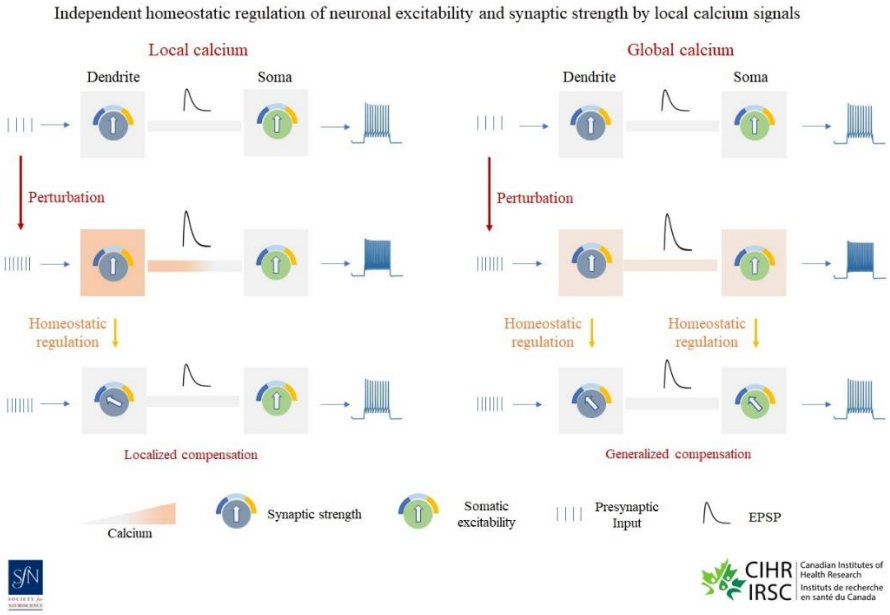
Topic: B.05. Synaptic Plasticity

Support: Foundation Grant from the Canadian Institutes of Health Research

Title: Independent homeostatic regulation of intrinsic excitability and synaptic strength by local calcium signals

Authors: *A. ROY[1], S. A. PRESCOTT[2];
[1]Univ. of Toronto, Univ. of Toronto, Toronto, ON, Canada; [2]Neurosciences and Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Neurons use action potentials (spikes) to process information. To function properly, neurons must regulate their spike output by adjusting the strength of their synaptic input and their responsiveness to that input. Homeostatic regulation of these two properties - synaptic strength and intrinsic excitability - has been the focus of significant research but certain issues remain unresolved; for instance, both properties utilize changes in intracellular calcium as a feedback signal to direct compensatory changes, but a single feedback signal cannot simultaneously regulate two properties independently. Notably, past computational studies have implicitly focused on global calcium changes because neurons were modeled as single, homogeneous compartments, yet separate studies have shown that calcium can change differently in different parts of a neuron, transiently reaching high concentrations within spatially restricted domains, unlike the subtler changes that occur globally. This insight leads to the hypothesis that local calcium signals can independently encode different error signals and there by support independent regulation of >1 properties. We tested our hypothesis in a simplistic two compartmental model with dendrite and soma containing calcium dependent homeostatic mechanisms. We showed that separate calcium signals allow the dendritic and somatic compartment to independently regulate synaptic strength and excitability, respectively, depending on the nature of the perturbation. The same could not be achieved using a global calcium signal. However, in real neurons due to ionic diffusion, calcium signals are never completely separate. Thus, we tested our hypothesis in a biophysically realistic neuron model with electrophysiological and calcium diffusion properties matching experimental recordings. Using this model, we showed that local calcium signals are separate enough to support independent homeostatic regulations in the neuron.



Disclosures: A. Roy: None. **S.A. Prescott:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.06

Topic: B.05. Synaptic Plasticity

Support: PP00P3_144816

Title: Rapid homeostatic modulation of transsynaptic nanocolumn rings

Authors: *P. MUTTATHUKUNNEL[1], P. FREI[1], S. PERRY[2], D. DICKMAN[2], M. MÜLLER[1]; [1]Univ. of Zurich, Zurich, Switzerland; [2]USC, Los Angeles, CA

Abstract: Robust neural information transfer relies on a delicate molecular architecture of chemical synapses and even subtle changes in the molecular organization of synapses may profoundly affect synaptic transmission and animal behavior. Neurotransmitter release is controlled by a specific arrangement of proteins within presynaptic active zones. How the specific presynaptic molecular architecture relates to postsynaptic organization, and how synaptic nano-architecture is transsynaptically regulated to achieve stable synaptic transmission remains enigmatic. Using STED microscopy, we here discovered that presynaptic nano-rings formed by the active-zone cytomatrix protein scaffold Bruchpilot (Brp) align with glutamate receptors (GluRs) nano-rings composed of ~6 GluR clusters at the *Drosophila* NMJ. Individual rings harbor ~5 transsynaptically-aligned Brp-GluR 'nanocolumns'. Similar transsynaptically-aligned nanocolumn rings are formed between Brp and the auxiliary GluR subunit Neto, or the presynaptic protein Unc13A and GluRs. Transsynaptic nanocolumn rings are partially masked by unaligned GluR clusters. Genetic manipulations of GluR subunits and Neto revealed a GluR subtype-specific nano-organization: While GluRs containing the GluRIIA subunit predominantly localize to nano-rings, GluRIIB clusters can be found inside and outside of the nano-rings. Interestingly, acute GluR impairment rapidly triggers the formation of new transsynaptic nanocolumns on the minute time scale during homeostatic plasticity. We reveal distinct phases of structural transsynaptic homeostatic plasticity, with postsynaptic GluR reorganization preceding presynaptic Brp modulation. Finally, the auxiliary GluR subunit Neto promotes structural and functional homeostatic plasticity. We are in the process of linking synaptic nano-architecture to synaptic physiology. Preliminary data suggest a correlation between nano-column number and the frequency of synaptic miniature events. Together, our results suggest that transsynaptic nanocolumns arrange in stereotypic rings that are rapidly modulated during homeostatic plasticity to stabilize synaptic efficacy.

Disclosures: P. Muttathukunnel: None. **P. Frei:** None. **S. Perry:** None. **D. Dickman:** None. **M. Müller:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

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Program #/Poster #: 609.07

Topic: B.05. Synaptic Plasticity

Support: DFG - SFB 1089 / A06 P02
BONFOR

Title: Temporal profile of presynaptic homeostatic plasticity

Authors: *J. SANTOS-TEJEDOR, D. DIETRICH, S. SCHOCH;
Bonn Univ. Med. Sch., Bonn, Germany

Abstract: Chemical synapses represent the key computational units of neuronal networks. Changes in the pattern of action potentials trigger alterations in the strength of synaptic transmission that contributes to the establishment of long- and short-term plasticity. In addition, neurons exhibit compensatory mechanisms to maintain neuronal network activity within a physiological range. This form of plasticity is defined as homeostatic plasticity (HP) and involves changes in both the pre- and postsynaptic terminals of a synapse. Presynaptic homeostatic plasticity (PHP) engages a complex web of signalling at the cytomatrix of the active zone (AZ) that contains a dynamic group of proteins involved in docking, priming and fusion of synaptic vesicles. Previous studies have demonstrated that PHP is induced after prolonged silencing periods of neuronal activity (48 hours *in vitro*) and is associated with structural changes at the AZ, for example increases in RIM nanocluster number. However, the exact molecular mechanisms underlying the induction of PHP are not well understood. Here, we studied the temporal profile of PHP establishment by investigating functional and structural changes in the presynaptic terminal after prolonged silencing of neuronal activity using tetrodotoxin (TTX) in primary cultured neurons. We performed super-resolution microscopy (dSTORM) to assess the number of nanoclusters and localisations of key active zone components. We found that the structural reorganization of the active zone and the functional changes associated with PHP, like increased influx in calcium and release of glutamate do not occur simultaneously but exhibit distinct temporal profiles. Taken together, our results indicate that the induction of PHP requires a tightly regulated stepwise reorganization of the presynaptic release machinery at the active zone.

Disclosures: **J. Santos-Tejedor:** None. **D. Dietrich:** None. **S. Schoch:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

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Program #/Poster #: 609.08

Topic: B.05. Synaptic Plasticity

Title: Evidence that protons mediate presynaptic homeostatic potentiation at the mouse neuromuscular junction.

Authors: S. TORRENCE, K. IMOMNAZAROV, ***C. A. LINDGREN**;
Biol., Grinnell Col., Grinnell, IA

Abstract: Synaptic homeostasis, which maintains constancy of synaptic transmission, is an essential function of the nervous system. A notable example of this process is presynaptic homeostatic potentiation (PHP), which occurs at the neuromuscular junction (NMJ) following the partial blockage of nicotinic acetylcholine receptors (nAChRs). In response to this partial blockage, quantal content (QC) increases and thereby restores synaptic transmission to normal levels. Previous research at the mouse NMJ indicates that, due to the activity of acid sensing ion channels (ASICs), a drop in pH of the synaptic cleft induces PHP. This suggests that protons are the retrograde signal that triggers an increase in QC. In this study, we further investigated the pH-dependency of PHP by using a strong HEPES buffer to increase the buffering capacity of the extracellular saline. This resulted in the abolishment of d-TC induced QC upregulation, indicating that a change in pH is necessary for PHP. Additionally, in experiments measuring QC at pH values of 7.0, 7.1, 7.15, and 7.2, we found that the magnitude of QC upregulation varies with pH and is maximized between pH 7.1 and 7.2. This increase in QC is consistent with the role of ASIC channels, which detect an increase in proton concentration. These observations suggest that PHP at the mouse NMJ depends on a drop in pH at the synaptic cleft. Given this, we sought to uncover what induces the drop in pH. Based on previous research, we speculate that the plasma membrane calcium ATPase (PMCA), which removes two protons from the synaptic cleft in exchange for a calcium ion, normally prevents the acidification of the cleft caused by the exocytosis of acidic synaptic vesicles. We further speculate that the activity of the PMCA in the postsynaptic membrane is activated by the calcium that enters the muscle through the nAChRs. Thus, when the nAChRs are partially blocked, less calcium enters, resulting in lower activity of the postsynaptic PMCA. This will reduce the buffering of protons and allow the pH in the synaptic cleft to drop. To test this idea, we injected carboxyeosin (CE), an inhibitor of the PMCA, into muscle cells. This induced an increase in QC, mimicking what happens during PHP and supporting a role for the PMCA.

Disclosures: S. Torrence: None. **K. Imomnazarov:** None. **C.A. Lindgren:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.09

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS111414

Title: Autocrine inhibition by a glutamate-gated chloride channel mediates presynaptic homeostatic depression

Authors: *D. K. DICKMAN, X. LI;
USC, Los Angeles, CA

Abstract: Homeostatic modulation of presynaptic neurotransmitter release is a fundamental form of plasticity that stabilizes neural activity, where presynaptic homeostatic depression (PHD) can adaptively diminish synaptic strength. PHD has been proposed to operate through an autocrine mechanism to homeostatically depress release probability in response to excess glutamate release at the *Drosophila* neuromuscular junction. This model implies the existence of a presynaptic glutamate autoreceptor. We systematically screened all neuronal glutamate receptors in the fly genome and identified the *glutamate-gated chloride channel* (*GluClα*) to be required for the expression of PHD. Pharmacological, genetic, and Ca^{2+} imaging experiments demonstrate that GluClα acts locally at axonal terminals to drive PHD. Surprisingly, GluClα localizes and traffics with synaptic vesicles to drive presynaptic inhibition through an activity-dependent anionic conductance. Thus, GluClα operates as both a sensor and effector of PHD to adaptively depress neurotransmitter release through an elegant autocrine inhibitory signaling mechanism at presynaptic terminals.

Disclosures: D.K. Dickman: None. **X. Li:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.10

Topic: B.05. Synaptic Plasticity

Support: NIH/NINDS Grant NS091546

Title: Evidence for retrograde homeostatic signaling transmitted by structural remodeling of the postsynaptic apparatus

Authors: *C. QIU[1,2], Y. HAN[1,2], P. GOEL[1,2], D. DICKMAN[2];
[1]Neurosci. Grad. Program, [2]Dept. of Neurobio., USC, Los Angeles, CA

Abstract: Synapses must be resilient to the challenges they confront during development, growth, disease, and aging. At the glutamatergic *Drosophila* neuromuscular junction (NMJ), acute pharmacological perturbations of postsynaptic glutamate receptors (GluRs) can be rapidly counteracted by enhanced presynaptic neurotransmitter release to restore synaptic strength, a process termed presynaptic homeostatic potentiation (PHP). Much is now known about the genes and mechanisms that enable the homeostatic increase in presynaptic glutamate release. However,

how retrograde PHP signaling is rapidly induced in the postsynaptic compartment remains enigmatic. Importantly, recent evidence suggests that perturbation of ionic flow through GluRs is insufficient induce PHP expression. Here, we combine molecular genetics, electrophysiology, and confocal and super-resolution imaging to provide evidence that conformational signaling, originating from postsynaptic GluR perturbation, is propagated throughout the postsynaptic apparatus. Preliminary data suggests this structural postsynaptic signaling is associated with the presynaptic hallmarks of PHP, including active zone remodeling and enhanced neurotransmitter release. Further, from a forward genetic screen we identify a key postsynaptic component that is targeted for conformational signaling to enable rapid PHP expression. Ongoing experiments are now focused on defining the role of synaptic activity, calcium signaling, and trans-synaptic interactions in rapid retrograde PHP signaling. Our working hypothesis is that conformational signaling initiated by GluRs is propagated throughout the postsynaptic compartment to transmit retrograde homeostatic information to presynaptic release sites, and that this synaptic dialogue can occur independently of synaptic activity.

Disclosures: **C. Qiu:** None. **Y. Han:** None. **P. Goel:** None. **D. Dickman:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

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Program #/Poster #: 609.11

Topic: B.05. Synaptic Plasticity

Support: NINDS Grant NS091546

Title: A glutamate receptor C-tail recruits CaMKII to suppress retrograde homeostatic signaling

Authors: ***S. PERRY**[1], Y. HAN[1], C. QIU[1], C. CHIEN[1], P. GOEL[1], S. NISHIMURA[1], M. SAJNANI[1], A. SCHIMD[2], S. J. SIGRIST[2], D. DICKMAN[1,2];
[1]USC, USC, Los Angeles, CA; [2]Inst. for Biology/Genetics, Freie Univ. Berlin, Berlin, Germany

Abstract: Presynaptic homeostatic plasticity (PHP) adaptively enhances neurotransmitter release following diminished postsynaptic glutamate receptor (GluR) function to maintain synaptic strength. While a lot is now known about the expression mechanisms of this fundamental form of plasticity, the postsynaptic induction process remains enigmatic. For over 20 years, it was hypothesized that diminished Ca^{2+} influx through postsynaptic GluRs reduces CaMKII activity to enable retrograde PHP signaling. Here, we have interrogated postsynaptic inductive signaling and the role of CaMKII at the *Drosophila* neuromuscular junction. First, we demonstrate that active CaMKII colocalizes with and requires the GluRIIA receptor subunit. Next, we used CRISPR mutagenesis to generate calcium-impermeable GluRIIA-containing receptors, revealing that both CaMKII activity and PHP induction are insensitive to reductions in postsynaptic Ca^{2+}. Rather, a short C-terminal domain encoded in the GluRIIA tail is necessary and sufficient to recruit active CaMKII to postsynaptic compartments. Finally, we use chimeric receptors to

demonstrate that the GluRIIA tail constitutively occludes retrograde homeostatic signaling by stabilizing active CaMKII. Thus, the physical loss of the GluRIIA tail is sensed, rather than reduced Ca^{2+} signaling, to enable retrograde PHP signaling, highlighting a unique, Ca^{2+}-independent control mechanism for CaMKII in homeostatic plasticity. Our ongoing work focuses on defining down-stream interaction partners of CaMKII and how PHP inductive signaling is ultimately conveyed to the presynaptic cell.

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Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.12

Topic: B.05. Synaptic Plasticity

Support: NIH/NINDS Grant NS111414

Title: Excess glutamate release triggers subunit-specific homeostatic receptor scaling

Authors: *N. L. TRAN, P. GOEL, Y. HAN, S. NISHIMURA, S. PERRY, M. SAJNANI, D. DICKMAN;
Dept. of Neurobio., USC, Los Angeles, CA

Abstract: Ionotropic glutamate receptors (GluRs) are targets for modulation in Hebbian and homeostatic synaptic plasticity and are remodeled by development, experience, and disease. Although much is known about activity-dependent mechanisms that regulate GluR composition and abundance, the role of glutamate itself in these processes is unclear. To determine how glutamate sculpts GluR receptive fields, we have manipulated synaptically released glutamate and generated precise CRISPR mutations in the two postsynaptic GluR subtypes at the *Drosophila* neuromuscular junction, GluRA and GluRB. We first demonstrate that GluRA and GluRB compete to establish postsynaptic receptive fields, and that proper GluR abundance and localization can be orchestrated in the absence of any synaptic glutamate release. However, excess glutamate release adaptively tunes postsynaptic GluR abundance, echoing GluR receptor scaling observed in mammalian systems. Unexpectedly, when GluRA vs GluRB competition is eliminated, excess glutamate homeostatically regulates GluRA abundance, while GluRB abundance is now insensitive to glutamate modulation. Finally, Ca^{2+} impermeable GluRA receptors are no longer sensitive to homeostatic regulation by glutamate. Thus, excess glutamate, GluR competition, and Ca^{2+} signaling collaborate to selectively target GluR subtypes for homeostatic regulation at postsynaptic compartments.

Disclosures: N.L. Tran: None. **P. Goel:** None. **Y. Han:** None. **S. Nishimura:** None. **S. Perry:** None. **M. Sajnani:** None. **D. Dickman:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.13

Topic: B.05. Synaptic Plasticity

Support: Swiss National Science Foundation grant PZ00P3_174018

Title: Homeostatic release depression counteracts positive AMPA receptor modulation

Authors: *K. KITA, M. MÜLLER, I. DELVENDAHL;
Dept. of Mol. Life Sci., Univ. of Zurich, Zurich, Switzerland

Abstract: The nervous system relies on an intricate network of neurons, synapses, and proteins. Yet, neural function is remarkably stable over time. There is evidence that homeostatic mechanisms counteract deviations of synaptic function and thus maintain synaptic efficacy within adaptive ranges. In the mammalian central nervous system, homeostatic potentiation of neurotransmitter release rapidly compensates for decreased glutamate receptor function on the minute time scale. If rapid presynaptic homeostatic plasticity is bidirectional remains unknown. Here, we combined pharmacological modulation of AMPA receptors and high-resolution electrophysiological analyses to investigate homeostatic depression of release at murine cerebellar mossy fiber to granule cell synapses. Acute wash-in of different AMPA receptor positive allosteric modulators (PAMs) consistently increased miniature excitatory postsynaptic currents (mEPSCs) in granule cells, indicating enhanced AMPA receptor function. Mossy fiber-stimulation evoked EPSCs, however, were differentially affected by PAMs: whereas cyclothiazide strongly enlarged EPSC amplitudes, EPSC amplitudes remained stable upon LY404187 or PEPA application. In the case of cyclothiazide, the increase in EPSC amplitude superseded the one in mEPSC amplitude, suggesting presynaptic release potentiation. Direct presynaptic whole-cell recordings revealed that action potential broadening likely underlies the release enhancement seen upon cyclothiazide treatment, consistent with previous reports. Conversely, increased mEPSC amplitude without apparent changes in EPSC amplitude after wash-in of LY404187 or PEPA implies homeostatic depression of release. Compensatory release depression evolved within minutes after PAM application and was accompanied by a decrease in functional release site number as assessed by quantal analysis. Our data thus establish that presynaptic homeostatic plasticity at mammalian central synapses is bidirectional and can counteract specific receptor perturbations on the minute timescale. By extension, the PAM type-dependent nature of presynaptic release depression suggests receptor perturbation-specific homeostatic signaling.

Disclosures: K. Kita: None. **M. Müller:** None. **I. Delvendahl:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.14

Topic: B.05. Synaptic Plasticity

Support: NIH NINDS 1R01NS112365-01A1
NSF CAREER IOS 1750199

Title: Using optical measurements of glutamate to detect a NMDA -dependent form of presynaptic homeostatic plasticity

Authors: *S. J. BERGERSON[1], M. B. HOPPA[2];
[2]Biol. Sci., [1]Integrative Neurosci. at Dartmouth, Hanover, NH

Abstract: The homeostatic mechanisms that regulate post-synaptic strength have been extensively studied. In contrast, relatively little attention has been paid to mechanisms of pre-synaptic homeostatic plasticity (PHP) and retrograde signaling, particularly in small synapses (<1 μm) of the central nervous system (CNS). Many diseases of aging in the CNS are associated with loss of synapses or chronic weakening of synaptic transmission. As such, a better understanding of the mechanisms of synaptic maintenance in this brain area is of critical importance. Measuring PHP is particularly challenging in this system because the presynaptic terminals are too small for whole-cell patch clamp measurements. Historically, to overcome this challenge, post-synaptic currents have been used as a proxy for measuring neurotransmitter release during synaptic transmission. Since impaired receptor function is the most common mechanism for initiating rapid forms of PHP, accurate measurements of neurotransmitter release are very challenging. Here we demonstrate a quantitative optical measurement of glutamate release from axons with a new form of fluorescent glutamate indicator (iGluSnFRv857). This indicator has both enhanced photostability and binding kinetics that avoid saturation and allow “quantal” measurements of multivesicular glutamate release. In cultured hippocampal neurons, we blocked classic glutamate receptors, NMDA and AMPA, individually. We found that selectively blocking AMPA receptors caused a rapid form of PHP that is only maintained with functional NDMA receptors. Selective AMPAR blockade caused a 24% increase in glutamate release that is highly sensitive to NMDAR activation (n = 13). Interestingly, this is likely not due to presynaptic NMDARs, as enhanced exocytosis occurs without any change in presynaptic calcium (n = 9). These data demonstrate that presynaptic strength is tightly temporally regulated by glutamatergic signaling through postsynaptic NMDARs.

Disclosures: S.J. Bergerson: None. **M.B. Hoppa:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.15

Topic: B.05. Synaptic Plasticity

Support: NIMH Grant MH1214878

Title: The loss of Shank3 perturbs mTOR protein interaction modules associated with signaling during homeostatic scaling

Authors: *D. WEHLE[1], W. E. HEAVNER[2], S. SMITH[1];
[1]Univ. of Washington, Seattle, WA; [2]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: While Autism spectrum disorder (ASD) is a heterogenous disorder with a wide array of causative mutations implicated, animal modeling studies of many implicated genes have identified shared mechanisms and deficits. One such common disfunction lies in a form of plasticity called homeostatic scaling, a non-Hebbian form of plasticity that allows a neuron to maintain its firing rate within a specific range of excitability. Another commonly observed deficit in ASD involves disruption of the PI3K-mTOR signaling cascade. The mTOR cascade has been implicated in many forms of plasticity including homeostatic scaling. To relate disruptions in mTOR signaling with disruptions in homeostatic scaling, we analyzed how changes in the mTOR protein interaction network (PIN) during scaling events in ASD model mice. We treated p16 to p20 cultured cortical neurons with tetrodotoxin (TTX) to induce upscaling and bicuculine (BIC) to induce downscaling. We then observed the response of a PIN composed of proteins involved in mTOR signal transduction using a novel Qualitative Multiplex Immunoprecipitation (QMI) antibody panel. We found extensive re-arrangement of protein complexes containing PI3K, mTORC1/2, TSC, FMRP and EIF4E/G, among others. The protein complexes altered by up- vs. down-scaling partially overlapped; both up- and down-scaling induced similar changes in a module of interactions involving the EIF4 complex, while down scaling produced protein network changes in a module that included TSC and mTORC1/2 complexes and up scaling induced a small set of unique interactions involving EIF4, AKT, p70S6K, and TSC. Next, we compared frontal cortical tissue from unmanipulated Shank3B KO and wildtype adult mice using QMI. We identified genotype-dependent differences in the mTOR PIN, including downregulation of a complex containing EIF4E and EIF4G, which suggested dysregulation of protein synthesis in Shank3 animals. Finally, we induced homeostatic scaling in Shank3B wildtype and mutant cortical cultures. The mTOR protein interaction modules of untreated mutant neurons more closely resembled the down-scaled state of wildtype neurons. EIF4G complex dysregulation was also observed during BIC treatment in the Shank3 mutant, further implicating the role of translation has in Shank3 related plasticity deficits. Overall, our results indicate that homeostatic scaling induces changes in distinct modules of mTOR and EIF4-related protein complexes. These mTOR protein interaction modules are dysregulated in the Shank3 knockout model, which may link molecular mTOR signaling deficits to the altered synaptic plasticity observed in this model of ASD.

Disclosures: D. Wehle: None. **W.E. Heavner:** None. **S. Smith:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

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Program #/Poster #: 609.16

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01NS117372

Title: An Extracellular Matrix Protease Controls Trans-synaptic Homeostatic Plasticity in Drosophila

Authors: *Y. CAI[1], P. PAGANELLI[1], J. CHEN[2], A. CHAKRABORTY[3], D. MORENCY[4], T. CUI[1], T. WANG[1,4];
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Abstract: Homeostatic plasticity is a form of synaptic regulation that stabilizes neural function. At the *Drosophila* neuromuscular junction (NMJ), when postsynaptic neurotransmitter receptor function is inhibited pharmacologically or genetically, an increase of presynaptic neurotransmitter release compensates for the postsynaptic perturbation so that the excitation is maintained at a stable level in the postsynaptic cell. This form of homeostatic regulation is termed Presynaptic Homeostatic Potentiation (PHP). PHP can be induced acutely by application of a glutamate receptor antagonist philanthotoxin or chronically by genetic deletion of glutamate receptor subunit GluRIIA. PHP is highly conserved across species, ranging from *Drosophila* to humans. In our previous studies, we found that a glial-secreted extracellular matrix (ECM) protein, Multiplexin, is critical for both acute induction and chronic maintenance of PHP. *Drosophila* Multiplexin, homologue to mammalian collagen XV/XVIII, is a soluble collagen in the ECM. Endostatin, the C-terminal domain of Multiplexin, can be proteolytically cleaved and released from Multiplexin. Intriguingly, Endostatin serves as a trans-synaptic signal to facilitate neurotransmitter release during PHP through presynaptic calcium channels. However, the identity of the protease that cleaves Multiplexin to release the signaling molecule Endostatin remains unknown. In this study, we investigate the function of ECM proteases in PHP. We identified an ECM protease that is necessary for PHP in an electrophysiology-based genetic screening. Using CRISPR genome editing, we generated a molecular null mutant allele of the identified protease. We systematically examined the function of the protease in PHP with electrophysiological, immunohistochemistry, and confocal-imaging methods. Our preliminary data suggest that this protease is necessary for both acute induction and chronic maintenance of PHP. In addition to our genetic evidence, we further validated our finding by a pharmacological inhibitor of the protease. We also demonstrated that this protease functions specifically in the postsynaptic muscle to control the trans-synaptic homeostatic plasticity using tissue-specific RNAi knockdown and rescue approaches. In summary, we have uncovered the identity and function of an ECM protease that controls presynaptic neurotransmitter release during homeostatic plasticity.

Disclosures: Y. Cai: None. **P. Paganelli:** None. **J. Chen:** None. **A. Chakraborty:** None. **D. Morency:** None. **T. Cui:** None. **T. Wang:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.17

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01NS085164

Title: Regulation of calcineurin signaling is essential for acute and chronic homeostatic synaptic plasticity in Drosophila

Authors: *N. ARMSTRONG, C. A. FRANK;
Univ. of Iowa, Iowa City, IA

Abstract: The ability of synapses to maintain physiological levels of evoked neurotransmission is essential for neuronal stability. Defective neurotransmission can result in a plethora of chronic disorders like forms of epilepsy and migraine or disturbed routine behaviors, like sleep. A variety of perturbations can disrupt neurotransmission, but synapses often compensate for disruptions to stabilize activity levels, using forms of homeostatic synaptic plasticity. Presynaptic homeostatic potentiation (PHP) is one such mechanism. In PHP, neurotransmitter release increases in response to challenges to the synapse, resulting in the maintenance of evoked neurotransmission. Prior work suggests that intracellular calcium signaling mediated by IP_3 receptors and Ryanodine receptors is required for the maintenance of PHP, but it is unclear what the mechanism is by which IP_3R and RyR - or downstream intracellular calcium signaling - function to maintain PHP. Calcineurin is a well conserved Ca^{2+}/calmodulin-dependent protein phosphatase that is found in most mammalian tissues but is expressed at high levels in the brain, and it has been shown to regulate ion channel function and trafficking, receptor trafficking, and neuronal plasticity. We used a combination of *Drosophila melanogaster* RNAi and overexpression fly lines, along with neuromuscular junction (NMJ) electrophysiology, synapse imaging, and pharmacology to test whether calcineurin and its regulators are necessary for the normal expression of acute and chronic PHP. We found that separately impairing either pre- or postsynaptically the regulator of calcineurin, *sarah* (*sra*), blocks PHP. Further examination of these tissue-specific data showed us that not only are increases and decreases in *sra* expression detrimental to PHP, but that the acute and chronic phases of PHP are functionally separable. Surprisingly, we also found that concurrent pre- and postsynaptic *sra* knockdown or overexpression led to much weaker or no blocks in either phase of PHP compared to single tissue impairments. Our current findings suggest that regulation of calcineurin signaling could be driving the downstream function of intracellular calcium signaling across multiple tissue types and over different temporal phases of PHP expression.

Disclosures: N. Armstrong: None. **C.A. Frank:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.18

Topic: B.05. Synaptic Plasticity

Support: NIH/NIND Grant NS091546

Title: A genetic screen of all Drosophila glutamate receptors identifies bao and ekar to be necessary for homeostatic plasticity

Authors: *C. CHIEN[1,2], Y. HAN[1,2], C. DONG[1], B. KIRAGASI[1], D. K. DICKMAN[1];
[1]Dept. of Neurobio., [2]Neurosci. Grad. Program, USC, Los Angeles, CA

Abstract: Glutamate receptors (GluRs) are major determinants of synaptic strength and plasticity. We have characterized the expression and functions of all 16 GluRs encoded in the *Drosophila* genome. In particular, we assessed roles for GluRs in synaptic growth, function, and plasticity at the *Drosophila* larval neuromuscular junction (NMJ), a model glutamatergic synapse. All major GluR subtypes are represented, including Kainate-, AMPA-, NMDA-, Glutamate-gated Chloride, and metabotropic GluRs. First, we find that representatives of each of the five GluR classes are expressed at NMJs: 9 are in motor neurons, 5 are in the postsynaptic muscle, while the remaining 2 GluRs are apparently not expressed in larval stages. Next, we generated unambiguous null mutations in all 16 GluRs using CRISPR/Cas9 mutagenesis and screened the 9 motor neuron GluRs for roles in NMJ growth and function. Surprisingly, each of the 9 GluR mutants viable and show no major defects in synaptic growth or baseline function, as assessed by electrophysiology. Finally, we have identified two GluR subunits, *bao* and *ekar*, not previously studied at the NMJ, to be required for the expression of homeostatic plasticity. Additional electrophysiological and genetic studies of these novel GluRs uncover unanticipated roles in controlling presynaptic function and plasticity.

Disclosures: C. Chien: None. **Y. Han:** None. **C. Dong:** None. **B. Kiragasi:** None. **D.K. Dickman:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.19

Topic: B.05. Synaptic Plasticity

Support: NIH/NINDS Grant NS091546

Title: Genetic dissection of the BLOC-1 complex identifies unique roles in synaptic growth, function and homeostatic plasticity in drosophila

Authors: *R. STARK, L. PORTER, D. DICKMAN;
Neurobio., USC, Los Angeles, CA

Abstract: The Biogenesis of Lysosome-Related Organelles Complex 1 (BLOC-1) is an octameric complex with putative roles in vesicle trafficking in a variety of tissues. This complex is highly conserved from flies to humans, with eight conserved genes: *pallidin*, *muted*, *dysbindin*, *snapin*, *blos1*, *blos2*, *blos3*, and *blos4*, where multiple BLOC-1 components have been implicated in disease, including schizophrenia. A subset of the BLOC-1 has been characterized in the *Drosophila* nervous system, where apparently shared roles in synaptic vesicle trafficking were identified. However, distinctive functions were observed in homeostatic plasticity, where only a subset of BLOC-1 was necessary. Recent innovation in CRISPR-Cas9 gene editing has enabled rapid and targeted mutagenesis in *Drosophila*, and we have created unambiguous null mutant alleles for each BLOC-1 component. Using this collection of mutations, we have systematically screened the BLOC-1 complex for roles in synaptic growth, function, and homeostatic plasticity. First, we characterized synaptic growth and structure at the *Drosophila* neuromuscular junction (NMJ). Next, we assessed BLOC-1 components for roles in neurotransmission using electrophysiology. Finally, we screened the entire BLOC-1 complex for roles in the rapid induction and sustained expression of presynaptic homeostatic potentiation (PHP). Preliminary data on this new collection of BLOC-1 mutations confirmed previous findings in which *dysbindin* and *snapin* were required for PHP, while *pallidin* and *blos1* were dispensable. Interestingly, one of the other components apparently has a unique and unanticipated essential function in development, while the remaining three components appear to be surprisingly dispensable for PHP expression. These preliminary findings suggest a working model in which specialized functions of BLOC-1 are needed for homeostatic plasticity and perhaps essential developmental processes, while the entire BLOC-1 may only be required to sustain synaptic vesicle pools under conditions of intense and sustain synaptic activity.

Disclosures: R. Stark: None. **L. Porter:** None. **D. Dickman:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.20

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS111414

Title: Using super resolution microscopy to characterize presynaptic nano-architecture at the Drosophila neuromuscular junction

Authors: *E. TCHITCHKAN, N. L. TRAN, D. K. DICKMAN;
Neurobio., USC, Los Angeles, CA

Abstract: The presynaptic terminal is a highly organized machine specialized for rapid, precise, and regulated neurotransmitter secretion. These functions are orchestrated by lipids and proteins arranged in a variety of sub-synaptic compartments, including the active zone (AZ), peri-active zone (PAZ), and synaptic vesicle pools. Conventional widefield and confocal imaging approaches have helped to establish the basic principles of how these conserved structures are organized. However, these imaging modalities are limited by the diffraction of light and cannot resolve the nano-architecture (tens of nanometers) of these structures, the scale at which neurotransmission is ultimately regulated. To circumvent this limit, super resolution imaging approaches, including STtimulated Emission-Depletion (STED) microscopy, have been developed. We have used STED imaging to define the structures and relationships of the AZ, PAZ, and synaptic vesicle pools at the *Drosophila* neuromuscular junction (NMJ). We find that an intricate and highly organized PAZ matrix envelope the AZs embedded in presynaptic boutons. Within the PAZ, we identify a distinctive “endocytic zone” that is closest to AZs and separate from cell adhesion proteins and other signaling components integrated within the PAZ. In addition, we find a minimum spacing rule between AZs of ~550 nm, which we hypothesize is the space necessary for efficient endocytic membrane trafficking following vesicle fusion at release sites. Finally, we find that while most synaptic vesicle markers are diffusely organized within the bouton, a subset of components appear to have unique and distinctive structural motifs at AZs, perhaps indicative of vesicle priming and fusion competence. Thus, highly organized nanostructures are arranged throughout presynaptic terminals that enable the rapid, regulated, and precise control of neurotransmitter release and recycling. This model provides a quantitative foundation for future studies to probe structural changes in presynaptic nanoarchitecture in mutant and disease states.

Disclosures: E. Tchitchkan: None. **N.L. Tran:** None. **D.K. Dickman:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.21

Topic: B.05. Synaptic Plasticity

Support: Simons Foundation Grant 551354
Brain and Behavior Research Foundation Grant 27792
NIH Grant 1R01NS117372

Title: Drosophila chd1 functions in glia for trans-synaptic homeostatic plasticity

Authors: *D. MORENCY[1], C. LOK[2], P. PAGANELLI[3], Y. CAI[4], T. CUI[3], T. WANG[4]; [1]Interdisciplinary Program in Neurosci., [2]Biol., [3]Pharmacol. and Physiol., Georgetown Univ., Washington DC, DC; [4]Pharmacol. and Physiol., Georgetown Univ., Washington, DC

Abstract: Autism Spectrum Disorders (ASD), epilepsy, and Intellectual and Developmental Disability (IDD) are highly prevalent and pervasive neurodevelopmental disorders. However, there are no FDA-approved drugs for the treatment of core symptoms in ASD or IDD and anti-epileptic drugs are ineffective for 30% of epilepsy patients, highlighting a need for a more comprehensive understanding of mechanisms underlying these disorders. Synaptic homeostatic plasticity has been identified as a commonly dysregulated feature in these disorders whereby a lack of synaptic stabilization may underlie core symptoms. The *Drosophila* neuromuscular junction (NMJ) is a powerful model synapse that displays robust homeostatic regulation of synaptic transmission which can be rapidly tested with electrophysiological methods and is translationally relevant to mechanisms in mice and humans. At this glutamatergic synapse, perturbation of postsynaptic receptors results in a compensatory increase of presynaptic neurotransmitter release to maintain the stable postsynaptic excitation which has been termed Presynaptic Homeostatic Potentiation (PHP). Our lab has previously identified epigenetic signaling in glia as novel and critical aspect of PHP induction and maintenance. Chromodomain Helicase DNA-binding Protein 2 (CHD2) is an epigenetic regulator implicated in human cases of ASD, epilepsy, and IDD. We found the *Drosophila chd1*, tightly conserved homologue of mammalian *chd2*, is required for PHP in an electrophysiology-based genetic screen. These findings have led to the current project to delineate the role *chd1* in PHP. Using genetic, electrophysiological, calcium imaging, and super-resolution imaging methods, we have elucidated how *chd1* deficiency impairs PHP. Current clamp recordings at the *Drosophila* NMJ in tandem with *Gal4 UAS* mediated knockdown and rescue experiments have demonstrated that *chd1* is required for PHP and plays differential roles in perineurial glia as well as muscle and motoneuron in acute and chronic PHP respectively. In our *chd1* mutants we have also identified deficits in the increases in calcium channel abundance, via super resolution imaging and calcium imaging, as well as in readily releasable vesicle pool sizes, via high frequency voltage-clamp stimulus trains, typically observed in PHP. Throughout our experiments, we have not observed sex differences therefore data presented includes both males and females. These studies are also translationally relevant, highlighting glial populations as a novel cell type for targeted interventions to ameliorate deficits in PHP in the context of ASD, epilepsy, and IDD.

Disclosures: D. Morency: None. **C. Lok:** None. **P. Paganelli:** None. **Y. Cai:** None. **T. Cui:** None. **T. Wang:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.22

Topic: B.05. Synaptic Plasticity

Support: R01EY025613

Title: Homeostatic regulation of visual cortical activity during a visually-guided naturalistic behavior

Authors: D. P. LEMAN[1], B. J. LANE[1,2], G. G. TURRIGIANO[1];
[1]Biol., Brandeis Univ., Waltham, MA; [2]Univ. of Missouri, Columbia, MO

Abstract: Neocortical firing rates have a lognormal distribution that spans multiple orders of magnitude and are remarkably stable across time. Individual neurons maintain their place in this distribution and return to the same basal rate following prolonged sensory perturbations, indicating individual firing rate set points. However, it is unclear whether firing rate set points remain stable in the face of salient experiences, such as learning. To investigate homeostatic regulation in a more naturalistic context, we use a paradigm where critical period rats learn to chase, capture, and consume live crickets. Rodents are opportunistic omnivores with an innate drive to hunt, but progressively improve their behavioral performance across multiple days of hunting. To test the importance of visual cortex in both the learning and performance of cricket hunting, we expressed excitatory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to parvalbumin-positive interneurons in V1 to shut down visual cortical activity acutely during hunting. Our data show that shutting down V1 decreased performance both during initial learning, and once animals had reached proficiency, indicating that V1 plays a critical role in hunting. Next, we measured how hunting perturbs the activity of individual V1 neurons using chronic, in vivo electrophysiology and behavioral recordings. We observe that firing rates in V1 are significantly modulated by hunting epochs compared to the inter-cricket interval between hunts. We also observe an increase in the percentage of neurons that respond to pursuit across multiple days of learning. Next, we sought to measure the long-term effects of learning on neuronal firing rates to examine if homeostatic mechanisms constrain mean firing rates, or if new set points may be established. Our preliminary data suggest that V1 neurons have stable baseline firing rates prior to hunting, but these dramatically shift away from baseline levels within hours post-hunting. These changes persist for at least 24 hours following hunting, suggesting that this salient experience causes long-term changes in neuronal excitability. Taken together, our preliminary data suggest that visual cortex plays a crucial role in the learning and performance of a naturalistic behavior. Furthermore, learning causes changes in neuronal responses not only during the behavior itself, but also on much longer timescales, indicating that learning induces a persistent change in excitability within V1. Our future work will provide insight into how Hebbian and homeostatic plasticity mechanisms interact to generate these dramatic learning-induced changes.

Disclosures: D.P. Leman: None. **B.J. Lane:** None. **G.G. Turrigiano:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.23

Topic: B.05. Synaptic Plasticity

Support: NIH, NINDS - R01NS065992

Title: Homeostatic plasticity in the developing barrel cortex following unilateral whisker deprivation

Authors: *A. LAKHANI, P. A. WENNER;
Sch. of Med., Emory Univ., Atlanta, GA

Abstract: Homeostatic plasticity represents a set of compensatory mechanisms that ensure the maintenance of activity levels following different kinds of perturbations. These mechanisms can involve synaptic compensations (homeostatic synaptic plasticity or HSP) and intrinsic compensations of membrane excitability (homeostatic intrinsic plasticity or HIP). Whisker deprivation is an *in vivo* perturbation that can trigger both HSP and HIP. The whisker-responsive barrel cortex allows for straightforward stimulation and behaviorally-relevant sensory deprivation (whisker trimming). An organizational advantage of the barrel cortex is that the somatotopically arranged barrels preferentially respond to the stimulation of one whisker (principal whisker or PW) on the contralateral side. While different methods of whisker deprivation produce distinct cortical responses, removing all whiskers on one side of the snout (unilateral whisker deprivation) for 7-14 days in P30 mice has been shown to trigger homeostatic plasticity in the barrel cortex, expressed as an increase in whisker-evoked responses in L4 and L2/3 regular spiking (RS) excitatory neurons. In order to characterize the homeostatic capacity at an earlier stage of development as the circuit is maturing, I have begun unilateral trimming of whiskers every other day from postnatal day 14-28 (PD 14-28). I then record whisker-evoked spiking using a 3x3 array of piezoelectric stimulators to stimulate the PW and surrounding whiskers at multiple velocities. I then examine spiking activity in the L4 and L2/3 cortex using a 32-channel NeuroNexus probe. Preliminary results suggest that the whisker-deprived mice show a homeostatic increase in whisker-evoked firing in L4 compared to non-deprived control mice. I will record at multiple time points within this deprivation period to observe how homeostatic plasticity mechanisms alter excitability over this developmental period. Autism mouse models have been associated with impaired homeostatic mechanisms, which are though to contribute to dysregulated activity levels in such neurodevelopmental disorders. Therefore, I will examine the possibility that homeostatic responses to whisker deprivation are altered in autism.

Disclosures: A. Lakhani: None. **P.A. Wenner:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.24

Topic: B.05. Synaptic Plasticity

Support: NIH REGXM

Title: Inactivity-induced synaptic homeostasis incorporates non-monotonic responses at inhibitory and excitatory synapses

Authors: *G. GRANT[1], S. D. SUN[2,1,3], D. LEVENSTEIN[4,1,3], R. W. TSIEN[1,3];
[1]Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [2]Cold Springs Harbor Lab., Cold Springs Harbor, NY; [3]Ctr. for Neural Sci., New York Univ., New York, NY; [4]Ctr. for Neural Sci., McGill Univ., Montreal, QC, Canada

Abstract: Neuronal plasticity is essential for brain function, underlying fundamental properties such as learning, memory, development, and recovery from injury. Neurons use two main forms of plasticity that allow both flexibility and stability: Hebbian plasticity which depresses lesser active circuit elements such as in long-term depression, while homeostatic plasticity boosts these neuronal elements derived of activity. Homeostatic plasticity is crucial in regulating the balance between neuronal excitation and inhibition, which ensures that activity is maintained within a physiological range through compensatory adjustments. Despite theoretical postulation that the mechanisms of homeostatic synaptic adaptations are dynamic, most experimental studies report on a single time point after the synapse has assumedly arrived at its new stable state. In this project, we utilize the widely accepted activity silencing using tetrodotoxin (TTX), to induce homeostatic responses at excitatory and inhibitory synapses. To characterize the time course of inactivity-induced synaptic homeostasis, we used whole-cell patch clamp to record miniature excitatory and inhibitory postsynaptic (mEPSCs and mIPSCs) currents from neuronal cortical cultures chronically treated with TTX for 0, 3, 6, 12, 24, 36, 48 and 72 hours and investigated changes in mini amplitude, frequency and kinetics. Contrary to previous reports, we found that the increase in mEPSC amplitude was strikingly non-monotonic, with observed peaks at 6 and 48 h of TTX treatment. mEPSC frequency also displayed a significant non-monotonic oscillatory change with TTX treatment. Additionally, mIPSC frequency was also significantly non-monotonic, with a distinct trough at 12 h of TTX treatment. Interestingly, we did not observe significant changes in mIPSC amplitude over the time period tested. Together, these data reveal intriguing differences in the homeostatic responses at excitatory and inhibitory synapses, which may contribute to homeostatic maintenance of excitatory and inhibitory balance following a chronic perturbation. Our results encourage further investigation of homeostatic regulation and the potential differences in molecular mechanisms at excitatory vs. inhibitory synapses.

Disclosures: G. Grant: None. **S.D. Sun:** None. **D. Levenstein:** None. **R.W. Tsien:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.25

Topic: B.05. Synaptic Plasticity

Support: 1RF1AG068063-01A1

Title: Endocannabinoid adaptation during homeostatic downscaling

Authors: *M. YE[1], S. K. MONROE[2], S. M. GAY[1], M. L. ARMSTRONG[3], D. YOUNGSTROM[4], F. URBINA[1], S. L. GUPTON[1], N. REISDORPH[3], G. H. DIERING[1]; [1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Duke Univ., Durham, NC; [3]Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [4]Natl. Inst. of Envrn. Hlth. Sci., Chapel Hill, NC

Abstract: Homeostatic regulation of neuronal activity in response to hyperexcitation is critical to maintaining network stability and functional signaling. Endocannabinoids (eCBs) are bioactive lipids synthesized in the post-synaptic compartments in response to neuronal activities to activate pre-synaptic cannabinoid receptor CB1. Therefore, the eCB system is well suited to coordinate pre- and post-synaptic activity. However, the role of the eCB system remains elusive in homeostatic adaptation to neuronal hyperexcitation. We induced hyperexcitation in cultured rat cortical neurons with chronic bicuculline treatment (BCC), known to induce homeostatic scaling down. We combined western blotting with targeted lipidomics to investigate biochemical changes during neuronal adaptation to hyperexcitation over 48 hours. Neurons adapt to hyperexcitation by downregulating total fatty acid amide hydrolase (FAAH), upregulating the endocannabinoid anandamide and related N-acylethanolamines (NAEs) and surface CB1. We used multi-electrode array and live-cell imaging (iGluSnFR) to investigate spontaneous synchronous network activities. We found that elevated endocannabinoid tone suppresses network activities during adaptation to hyperexcitation, and CB1 signaling supports network events at baseline. Our data shows that coordinated adaptation of the eCB system supports homeostatic plasticity.

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Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.26

Topic: B.05. Synaptic Plasticity

Support: NIH GRANT12982452

Title: Homeostatic plasticity in preganglionic and postganglionic sympathetic neurons of the chick embryo

Authors: *A. RATLIFF, G. OLMEDO, D. PEKALA, P. WENNER;
Emory Univ., Atlanta, GA

Abstract: Homeostatic plasticity (HP) describes several mechanisms that a cell can use to maintain a biologically relevant level of output in response to changing input. HP mechanisms

can include compensatory changes in synaptic strength and intrinsic cellular excitability. These mechanisms have been identified throughout the nervous system from developing spinal motoneurons (MNs) to mature cortical neurons.
Embryonic MNs have been shown to utilize both synaptic and intrinsic mechanisms in response to chronic reductions of synaptic input as the circuit is being established. In the sympathetic nervous system (SNS), regulation of activity is imperative for healthy organ function and is under tight homeostatic control. This system has long been thought to be regulated by higher level feedback from the brainstem. However, HP at the level of sympathetic pre-ganglionic neurons (SPNs) and post-ganglionic neurons (PGNs) of the SNS has largely been ignored.
To test the homeostatic capacity of these neurons during circuit development, we are examining cellular responses to synaptic blockade in the embryonic chick. We have determined that the synaptic connection from SPNs to PGNs was immature at embryonic day 10 (E10) but can drive spiking in PGNs from E13 onward. These signals were reduced after application of ganglionic nicotinic blocker Hexamethonium (Hex). Therefore, E13 embryos were treated with either vehicle (H_2O) or Hex *in ovo* to block the sole input of these neurons for 48 hours. The acute and chronic behavioral effect of Hex blockade was measured using both video and electrical recordings *in ovo*. Intracellular whole cell patch recordings of PGNs were performed on these embryos at E15. At present, Hex-treated PGNs do not appear to be more excitable than vehicle-treated neurons. These results suggest that the E15 PGNs may not possess mechanisms to homeostatically alter their excitability in response to a chronic reduction in synaptic activity.
Next, SPNs were inspected for evidence of HP mechanisms. Previous work has shown that blocking GABAergic transmission for 2 days *in ovo* (E8-10) triggers a form of HP called synaptic scaling in MNs, which was mediated by intracellular chloride accumulation measured with a genetically-encoded chloride indicator. To test whether SPNs experience synaptic scaling in a similar fashion, we are repeating these experiments, focusing on SPNs. Preliminary results suggest that SPNs show increases in intracellular chloride concentration, suggesting they do exhibit synaptic scaling. This would be the first data of its kind to indicate that the SNS can respond to changes in synaptic signaling during embryonic development using HP mechanisms.

Disclosures: **A. Ratliff:** None. **G. Olmedo:** None. **D. Pekala:** None. **P. Wenner:** None.

Poster

609. Homeostatic Plasticity: Mechanisms, Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 609.27

Topic: B.05. Synaptic Plasticity

Support: NIH Grant 1R01NS114514

Title: Reduced GABA-ergic neurotransmission stabilizes respiratory motor output following chronic inactivity during hibernation in bullfrogs

Authors: *S. E. SAUNDERS, J. M. SANTIN;
Biol., Univ. of North Carolina at Greensboro, Greensboro, NC

Abstract: Neural systems must maintain function in the presence of a changing environment. One mechanism by which neurons accomplish this is through activity-dependent regulation of excitation and inhibition. American bullfrogs hibernate in ice-covered ponds, and during this period, the neural circuits that produce breathing are completely silent. We previously showed that compensatory upregulation of excitatory synaptic strength acts to boost respiratory motor drive to ensure breathing can restart to meet metabolic demands after months of inactivity. Given that compensatory changes in synaptic inhibition play a role in stabilizing network activity, we hypothesized chronic respiratory motor inactivity during hibernation would decrease inhibition in the respiratory network. In the intact respiratory circuit, blockade of GABA-A receptors typically disrupted respiratory motor output during the first 10 minutes of drug perfusion, whereby hyperexcitable motor output occurred in 4 out of 5 control preparations. However, none of the brainstem preparations (0/4) from frogs following hibernation exhibited chaotic respiratory motor output after blocking GABA-A receptors. Therefore, GABA-A receptor tone of the respiratory network is decreased following inactivity. To identify mechanisms that underlie the change in GABA-ergic tone, we studied miniature inhibitory postsynaptic currents (mIPSCs) from identified respiratory motoneurons in brainstem slices. Frogs following respiratory motor inactivity had decreased average GABA-A miniature inhibitory postsynaptic current (mIPSC) charge transfer ($p=.0118$). Furthermore, the amplitude distribution of individual mIPSCs from chronically inactive motoneurons was left shifted (reduced) compared to controls ($p<0.0001$), due to a weakening of synaptic strength across the 20-80th percentile of the distribution. These results indicate that inhibitory drive onto respiratory motoneurons decreases to regulate activity of the respiratory network following chronic inactivity. Overall, our data indicate that both decreased synaptic inhibition and enhanced synaptic excitation promote stable network output in the context of ecologically-relevant inactivity in vivo.

Disclosures: S.E. Saunders: None. **J.M. Santin:** None.

Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 610.01

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS083085

Title: Activity-dependent localization and de novo protein synthesis of Camk2a mRNAs in dendritic spines of hippocampal neurons

Authors: *D. HWANG, S. DAS, R. H. SINGER;
Cell Biol., Albert Einstein Col. of Med., Bronx, NY

Abstract: Calcium-mediated activation of Calcium/calmodulin-dependent protein kinase type II (CaMKII) is sufficient for inducing spine-specific structural long-term potentiation (sLTP) and also necessary for establishing long-term synaptic plasticity. Hence, the synaptic tagging and capture hypothesis postulates that activated CaMKII serves as a molecular tag that potentiates downstream molecular events resulting in long-lasting synaptic changes. CaMKII within dendritic spines is activated rapidly - within a minute - upon stimulation and persists during the establishment of long-term synaptic plasticity. However, the molecular events that ensure availability of CaMKII within stimulated spines and maintain a spine-specific pool of CaMKII during synaptic plasticity are unclear. We hypothesized that CaMKII becomes available to the spines via activity-dependent *de novo* local protein synthesis: *Camk2a* mRNAs may localize to or near stimulated spines and undergo rounds of translation during the maintenance phase of synaptic changes. To test this hypothesis, we developed a mouse model in which the endogenous *Camk2a* mRNA, which encodes the major α subunit of the CaMKII holoenzyme, was genetically tagged at the end of the 3' UTR with an array of RNA stem loops, and single mRNA molecules were orthogonally detected by fluorescently-labeled capsid proteins. Live-cell imaging of hippocampal neurons from these mice revealed that upon stimulation, *Camk2a* mRNAs were recruited to stimulated spines on a timescale of seconds. To assess whether *Camk2a* mRNAs within the spines are indeed locally translated, we further devised a CRISPR/Cas9-mediated knock-in of the SunTag epitope array at the 5' coding end of *Camk2a* mRNA. In support of our hypothesis, *de novo* translation of spine-localized *Camk2a* mRNAs was detected and persisted for minutes. We thereby propose that activity-dependent localization of *Camk2a* mRNAs generate a spine-specific pool of CaMKIIα, which supplies newly synthesized proteins to stimulated dendritic spines during the maintenance phase of long-lasting synaptic changes.

Disclosures: D. Hwang: None. **S. Das:** None. **R.H. Singer:** None.

Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 610.02

Topic: B.05. Synaptic Plasticity

Support: NIH grant NS034007
NIH grant NS122316

Title: Gadd34 mediates bdnf-induced increases in neuronal de novo protein synthesis and memory

Authors: *M. M. OLIVEIRA[1], M. K. ELDER[1], M. MOHAMED[1], K. BANEGAS-MORALES[1], H. T. EVANS[1], J. ALAPIN[1], E. H. LU[1], M. MAMCARZ[1], E. A. N. GOLHAN[1], N. NAVRANGE[1], S. V. KALAVAI[1], S. CHATTERJEE[2], T. ABEL[2], E. KLANN[1];
[1]New York Univ., New York Univ. Ctr. For Neural Sci., New York City, NY; [2]Dept. of Biol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Several types of translational control at the initiation step are known to be required increases neuronal protein synthesis that are required for memory consolidation. Moreover, regulation of translation initiation via the dephosphorylation of the eukaryotic initiation factor 2α (p-eIF2α) has emerged as a hub that integrates neuronal activity and translation during memory consolidation. eIF2α is the regulatory subunit of eIF2 that when phosphorylated inhibits general protein synthesis. In the hippocampus, eIF2α is dephosphorylated following learning, which is required to increase protein synthesis that facilitates the transition of short- to long-term memory. However, the mechanism by which eIF2α dephosphorylation occurs and how it is regulated by neuronal activity is unknown. We hypothesized that neuronal stimulation promotes increased protein synthesis by elevating the expression of GADD34, a scaffolding protein that facilitates eIF2α dephosphorylation by placing it into proximity to protein phosphatase 1. Here, we show that stimulating primary neurons with brain-derived neurotrophic factor (BDNF) promotes an increase in GADD34 protein levels within one hour of stimulation. In addition, we have found that this increase is translationally regulated, and independent of the canonical integrated stress response (ISR) pathway through which GADD34 is normally induced. Moreover, we observed that BDNF increases the physical interaction between GADD34 and eIF2α in both soma and dendritic compartments, and that GADD34 mediates BDNF-induced increases in protein synthesis. Finally, we have found that GADD34 is crucial for hippocampal-dependent long-term memory, which suggests that it mediates the increase in neuronal protein synthesis during memory consolidation. In summary, our results provide evidence of a novel GADD34-eIF2α pathway to increase neuronal protein synthesis in response to activity and learning. This work was supported by NIH grants NS034007 and NS122316 (E.K.)

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 610.03

Topic: B.05. Synaptic Plasticity

Support: R37 NS035546
R35 NS127314

Title: Regulation of the neuronal proteome by translation initiation factor eIF3 modulates excitation-inhibition balance

Authors: ***S. BLAZIE**[1], S. TAKAYANAGI-KIYA[2], Y. ZHAO[2], B. HU[2], Y. JIN[3];
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Abstract: Modulation of protein synthesis through mechanisms of translation control plays important roles in regulating neuronal function. Brain disorders featuring neuronal activity imbalances often coincide with dysregulated protein synthesis, underscoring the need to understand which factors execute neuronal translation control and how the factors are tethered to neuronal activity changes.Protein expression is primarily regulated during translation initiation by six eukaryotic initiation factors (eIF1-6), which ultimately control the efficiency of ribosome engagement with the mRNA start codon. eIF3 is the largest factor, consisted of thirteen unique subunits. In addition to the essential roles of most eIF3 subunits in general translation, recent work has shown that they also mediate specialized functions that modulate translation efficiency in particular biological contexts. Dysregulation of eIF3 subunits is also observed among neurological disorders, suggesting that eIF3 additionally manages aspects of neuronal translation control that are so far not defined. Through genetic and biochemical dissection of *C. elegans* EIF-3.G(eIF3g), an RNA-binding subunit of eIF3, we found that EIF-3.G remodels the neuronal proteome in response to elevated cholinergic motor neuron (ACh-MN) activity. We developed a neuron type-specific translatome profiling approach and found that EIF-3.G selectively regulates translation of ~200 mRNAs that encode critical regulators of neuronal activity. This regulatory function of EIF-3.G is conveyed by its poorly understood zinc finger domain. Disruption of the zinc finger strongly suppresses seizure-like behavior in *C. elegans* mutants expressing a hyperactive acetylcholine receptor without affecting general translation (Blazie et al., 2021; DOI: 10.7554/eLife.68336). As little is known about eIF3 function in the nervous system, we employed a genetic screening approach to identify functional partners. Here, we present evidence that EIF-3.G regulates ACh-MN activity through a cold-shock domain protein called LIN-66. We show that LIN-66 directly interacts with EIF-3.G to modulate the translation of EIF-3.G mRNA targets. Our data introduce a novel translation control pathway in neurons, whereby specific interactions between the EIF-3 complex and auxillary factors converge to sculpt the neuronal proteome in response to activity changes.

Disclosures: S. Blazie: None. **S. Takayanagi-Kiya:** None. **Y. Zhao:** None. **B. Hu:** None. **Y. Jin:** None.

Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 610.04

Topic: B.05. Synaptic Plasticity

Title: Transcriptomic mapping uncovers Purkinje neuron plasticity driving learning

Authors: *X. CHEN[1], Y. DU[2], G. BROUSSARD[3], M. KISLIN[3], S. DIETMAN[1], S. WANG[3], X. ZHANG[2], A. BONNI[1];
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Abstract: Cellular diversification is critical for specialized functions of the brain including learning and memory. Single-cell RNA sequencing facilitates transcriptomic profiling of distinct major types of neuron, but the divergence of transcriptomic profiles within a neuronal population and their link to function remain poorly understood. Here we isolate nuclei tagged in specific cell types followed by single-nucleus RNA sequencing to profile Purkinje neurons and map their responses to motor activity and learning. We find that two major subpopulations of Purkinje neurons, identified by expression of the genes Aldoc and Plcb4, bear distinct transcriptomic features. Plcb4^{+}, but not Aldoc^{+}, Purkinje neurons exhibit robust plasticity of gene expression in mice subjected to sensorimotor and learning experience. *In vivo* calcium imaging and optogenetic perturbation reveal that Plcb4^{+} Purkinje neurons have a crucial role in associative learning. Integrating single-nucleus RNA sequencing datasets with weighted gene co-expression network analysis uncovers a learning gene module that includes components of FGFR2 signaling in Plcb4^{+} Purkinje neurons. Knockout of Fgfr2 in Plcb4^{+} Purkinje neurons in mice using CRISPR disrupts motor learning. Our findings define how diversification of Purkinje neurons is linked to their responses in motor learning and provide a foundation for understanding their differential vulnerability to neurological disorders.

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

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Program #/Poster #: 610.05

Topic: B.05. Synaptic Plasticity

Support: 5R01MH119541
1R21MH127734
5R21DA039417
5R21DA041547

Title: Experience dependentexpression and dendritic targeting of lncRNA D17rik in CA1 neurons constrains encoding of contextual fear memory

Authors: I. ESPADAS[1], J. WINGFIELD[1], E. GRINMAN[1], B. RAVEENDRA[1], I. GHOSH[2], K. BAUER[3], V. RANGARAJU[2], M. KIEBLER[3], ***S. PUTHANVEETTIL**[1];
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Abstract: Specific changes in the gene transcription are a pre-requisite for long-term memory (LTM) storage. However, it remains largely unknown whether transcriptional changes in long noncoding RNAs (lncRNAs) are associated with learning and whether these changes mediate the consolidation of long-term memories (LTM). Here we report the identification of lncRNA

D17rik from unbiased analysis of gene expression changes in microdissected CA1 neurons of mouse hippocampus following contextual fear conditioning training (CFC). LncRNA D17rik becomes enriched in CA1 neurons but not in CA3 or dentate gyrus neurons in fear-conditioned animals compared to context alone or immediate shock controls. The loss of function of D17rik in CA1 impairs encoding but not acquisition, recall and extinction of CFC memory. MS2 based RNA tracking, time lapse quantitative live imaging and glutamate uncaging studies show that D17rik is transported to dendritic spines through a molecular motor dependent mechanism and that D17rik constrain dendritic spine morphology. Importantly, proteomics, RNAseq and miRNAseq revealed that several mRNAs, miRNAs, and proteins involved synaptic plasticity interacts with D17rik. These results establish region specific regulation, and memory phase specific role of a novel lncRNA in hippocampus and provide key insights into its mechanism of function.

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

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Topic: B.05. Synaptic Plasticity

Support: NIH R01 DA032708
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NIH (UL1 TR001450 to M.T.

Title: A long non coding enhancer RNA regulates the immediate early gene NPAS4 to control maladaptive reward-related behaviors induced by drugs or stress

Authors: *R. AKIKI[1], R. CORNBROOKS[1], K. MAGAMI[1], E. BRACE[1], P. MACE[1], B. W. HUGHES[1], N. KOIKE[2], S. BERTO[1], C. W. COWAN[1], M. TANIGUCHI[1];
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Abstract: Exposure to pathological stimuli (e.g., addictive drugs and stress) produces maladaptive behavioral changes that disrupt brain reward function and compromise mental health. Precise regulation of gene expression is important for adaptive brain function, and transcriptional mechanisms contribute to maladaptive changes in brain function that contribute to neuropsychiatric symptoms produced by stress and substance abuse. The neuronal activity-dependent, immediate early gene, NPAS4 (neuronal Pas domain containing protein 4) plays an

essential role in multiple brain regions, including the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC), to influence behavioral adaptations that result from cocaine exposure or chronic stress. NPAS4 is required in the NAc for the development of drug seeking-like behavior in the cocaine conditioned place preference assay (Taniguchi et al, *Neuron* 2017), and it is required in the mPFC during chronic social defeat stress (CSDS) to produce anhedonia-like behaviors, including reduced sucrose preference and altered motivation to self-administer sucrose. Here we show that stress and drugs of abuse induce NPAS4 expression through a process requiring a novel, non-annotated, lnc-enhancer RNA (lnc-eRNA) transcribed from the enhancer region of *Npas4 (Npas4*^{eRNA}) in the NAc and PFC *in vivo*. We find that brain region-specific reduction of the *Npas4*^{eRNA} disrupts Npas4 mRNA transcription and it is necessary for both drug and stress-induced behavioral changes. Ongoing studies are examining the mechanisms by which *Npas4*^{eRNA} regulates the activity-dependent induction of *Npas4*^{mRNA} and have identified a novel molecular structure at the NPAS4 enhancer. Together our studies reveal a novel role for a lnc-eRNA in the development of maladaptive behaviors produced by both addictive substances and chronic stress.

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

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Program #/Poster #: 610.07

Topic: B.05. Synaptic Plasticity

Support: NIH Grant MH111464-03
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Title: Essential role for the neurodevelopmental disorder-linked gene, MEF2C, in GABAergic neuron function and neurotypical social behavior

Authors: *Y. CHO, E. TSVETKOV, T. R. SATO, S. BERTO, A. ASSALI, C. W. COWAN; Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: The MEF2 (Myocyte Enhancer Factor 2) family of transcription factors regulate gene expression controlling cell differentiation and synapse development. Loss-of-function mutations or deletions of the *MEF2C* gene cause a neurodevelopmental disorder, termed *MEF2C* Haploinsufficiency Syndrome (MCHS). MCHS includes symptoms of autism spectrum disorder (ASD), intellectual disability, seizures, and motor and sensory abnormalities. MEF2C is highly expressed in excitatory forebrain neurons and GABAergic neurons, but the effects of MEF2C hypofunction in GABAergic cells for MCHS-like phenotypes is unknown. To study the role of MEF2C in GABAergic cell populations during mouse development, we bred *Vgat* (vesicular

GABA transporter)-Cre mice, which express Cre recombinase broadly in early developing GABAergic neurons, with a floxed *Mef2c* loss-of-function mouse to create offspring that are GABAergic cell-specific *Mef2c* heterozygous mutants (*Mef2c*$^{fl/+}$;*Vgat*-Cre or *Mef2c* cHet^{Vgat}). We then subjected the *Mef2c* cHet^{Vgat} and littermate control mice to a battery of tests measuring MCHS-relevant phenotypes, including social preference, learning and memory, and sensory sensitivity. Interestingly, *Mef2c* cHet^{Vgat} female, but not male, mice displayed significant deficits in sociability. Using additional cell type-specific Cre driver lines, we are in the process of further defining the relevant GABAergic cell subtypes relevant to sociability deficits. Moreover, in the *Mef2c* cHet^{Vgat} female mice, we observed significant alterations in excitatory/inhibitory balance and physiological properties of GABAergic interneurons in the prefrontal cortex (PFC), a brain region linked to social behavior deficits in multiple mouse models of ASD. Using a single-cell RNA-Seq approach, we also observed significant differentially expressed genes (DEGs) in multiple GABAergic cell types in PFC, and the most affected GABAergic population showed significant DEG enrichment for risk genes linked to ASD, schizophrenia, and intellectual disability. Together, our findings reveal an important, sex-specific role for MEF2C in GABAergic cells in neurotypical social behavior in mice.

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 610.08

Topic: B.05. Synaptic Plasticity

Support: K99DA04742601A1

Title: Stress Cues Exposure Induced Excitatory Plasticity in the Pentapartite Synapse

Authors: *C. GARCIA-KELLER, M. MEYERINK;
Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Stress Cues Exposure Induced Excitatory Plasticity in the Pentapartite SynapseMeyerink M and **Garcia-Keller C**Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin, USAEpidemiological studies indicate that acute life threatening events increases the incidence of post-traumatic stressdisorder (PTSD), and a diagnosis for PTSD carries 30-50% comorbidity with substance use disorder (SUD). Presentation ofdrug-associated cues evoke synaptic potentiation in the Pentapartite synapse, including pre and postsynaptic neurons,astrocytes, microglia and the extracellular matrix (ECM). Given the overlap between the enduring adaptations producedby stress and drug use, we hypothesized that animals exposed to a stress-conditioned stimulus (stress-CS) elicit synapticpotentiation and coping responses such as defensive burying behavior (DB). Moreover,

MSNs constitute 90-95% of theneurons in the Nucleus Accumbens Core (NAcore) and are chemically coded into two subtypes that selectively expresseither D1 or D2 dopamine receptors. Generally, D1-MSN activation promotes behavior, and D2-MSN activation inhibitsbehavior. Therefore, we hypothesized that stress cue-induced synaptic potentiation will occur in D1-MSNs. We found thatanimals exposed to stress-CS induced an increased aversive response and is associated with synaptic potentiation inNAcore quantified as: increased spine head diameter and density, increased metalloprotease-9 activity that catalyzesproteins from the ECM, increased microglia activation and astrocyte retraction from synapsis compared to control animals.Then, by performing single cell Ca^{2+} dynamics in D1- and D2-MSNs of freely moving rats, using a cre-dependent Ca^{2+}indicator, we observed that there is a dynamic dichotomy in the activation of the accumbal cell population. Repeatedexposure to stress-CS suggested a behavioral phenotype differentiation of susceptible and resilient groups. Preliminarydata indicates that the dynamic dichotomy of activity between D1- and D2-MSNs could be the underlying cause of thebehavioral phenotype observed. Specifically, we observed that susceptible rats have enhanced Ca^{2+} dynamics in D1-MSNsand resilient rats showed enhanced Ca^{2+} dynamics in D2-MSNs. Insights into how stress, stress cues and their relationshipwith drug-related activity Ca^{2+} dynamics provide a foundation for understanding the circuit-level stress and addictionpathogenesis.Keywords: stress disorder, calcium imaging, medium spiny neurons, nucleus accumbens, synaptic plasticity.**Support: K99DA047426-01A1** (CGK)**Disclosures: M.M:** None**, C.G.K.:** None,

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 610.09

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01 DA032708
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Title: Npas4 controls cell type-specific circuit adaptations underlying drug seeking behavior

Authors: B. W. HUGHES, E. TSVETKOV, B. M. SIEMSEN, K. K. SNYDER, R. AKIKI, D. J. WOOD, R. D. PENROD, M. D. SCOFIELD, S. BERTO, M. TANIGUCHI, ***C. W. COWAN**; Med. Univ. of South Carolina, Charleston, SC

Abstract: Re-exposure to diffuse and discreet cues associated with active drug use can trigger relapse in individuals recovering from substance use disorder (SUD). Relapse-like behavior is promoted in the nucleus accumbens (NAc) by the preponderant activation of D1 dopamine receptor-expressing medium spiny neurons (D1-MSNs), while it is generally impeded by D2

dopamine receptor-expressing MSN (D2-MSN) stimulation. However, the molecular mechanisms controlling the activation balance of D1-MSNs and D2-MSNs during relapse-like drug seeking remain unknown. We show here that the activity-regulated transcription factor, Neuronal PAS Domain Protein 4 (NPAS4), plays an essential role in this process. First, using a new NPAS4-TRAP (Targeted Recombination in Active Populations) mouse combined with chemogenetics, we found that NPAS4-positive neurons during cocaine conditioning, which are mostly D1- and D2-MSNs of the NAc, are required for expression of cocaine conditioned place preference (CPP). In addition, using cell type-specific manipulations, we discovered that NPAS4 in D2-MSNs, but not D1-MSNs, is required for both cocaine CPP and cue-reinstated drug seeking following cocaine self-administration (SA). Moreover, single nuclei RNA-sequencing (snRNA-seq) of NAc tissues from drug-experienced mice revealed that NPAS4 regulates numerous D2-MSN genes linked to cocaine exposure, dopamine regulation, and synaptic plasticity. Finally, NPAS4 in NAc D2-MSNs blocked cocaine experience-dependent strengthening of glutamatergic prefrontal cortical (PFC) inputs onto those neurons, a NAc afferent that is required for cued drug-seeking behavior, and NPAS4 blocked a cocaine conditioning-induced increase in dendritic spines on D2-MSNs. Together, our findings reveal a novel, cell type-specific function for NPAS4 to enable relapse-like behavior by selectively preventing drug experience-dependent potentiation of the PFC->$NAc^{D2\text{-}MSN}$ circuit. These data provide fundamental knowledge about the regulation of MSN-subtype E/I balance by NPAS4 during drug reward experiences, which will lead to a better understanding of its molecular contribution to relapse vulnerability.

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 610.10

Topic: B.05. Synaptic Plasticity

Support: R01MH121979

Title: Coordinated plasticity across multiple neuronal circuit layers via top-down gating of dendritic calcium spikes and behavioral timescale synaptic plasticity (BTSP)

Authors: A. PEDDADA, A. GALLONI, ***A. MILSTEIN**;
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Abstract: Here we consider a central problem in biological learning - how does information about the outcome of a decision or behavior modify the right synapses in the right neurons across multiple layers of neuronal circuitry to improve future performance? The standard approach to supervised learning in multi-layer artificial networks is to perform direct gradient descent to minimize the error in the output of the network with respect to its synaptic weights. Artificial neuronal units are typically implemented as simple point source integrators of synaptic input, and single units are permitted to form both excitatory and inhibitory synapses onto downstream neurons. In contrast, biological neurons are comprised of multiple dendritic compartments, and biological neuronal circuits typically contain separate populations of excitatory neurons (with only positive outgoing connections) and inhibitory neurons (with only negative outgoing connections). However, biological networks are typically thought to implement unsupervised Hebbian learning rules, which do not prescribe a mechanism for learning to be coordinated across multiple layers. Recent work has demonstrated that neurons with extended dendrites may have special features that are advantageous for supervised learning. In addition to feedforward connections that transmit sensory information "bottom-up" from the periphery, biological neurons also send information backwards along a hierarchy of neuronal circuitry through extensive "top-down" feedback connections. Interestingly, these feedback connections typically impinge on distal neuronal dendritic compartments, and when these feedback inputs exceed a local threshold in the distal dendrite, an event called a "dendritic calcium spike" is emitted. This event both increases the output of a neuron, and induces a non-Hebbian form of plasticity called "behavioral timescale synaptic plasticity" (BTSP). BTSP induces large changes in synaptic weights at both feedforward and feedback inputs and can cause a neuron to become selective for novel stimulus features after as little as a single stimulus presentation, a phenomenon called "one-shot learning". We demonstrate that regulation of dendritic spiking and plasticity by top-down feedback signals can effectively coordinate plasticity across multiple layers of a neuronal network during pattern recognition learning. We will show analysis of hidden feature representations in networks trained with top-down gating of dendritic plasticity, and will compare to networks trained with standard backpropagation or Hebbian learning rules.

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Poster

610. Transcriptional and Translational Control of Synaptic Plasticity and Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 610.11

Topic: B.05. Synaptic Plasticity

Support: BSF grant 2017342
ISF grant 2354/19
MOA Fellowship (O.R.)

Title: Experience-induced genomic enhancers tune visual processing in the adult cortex

Authors: *E. ZOHAR[1], O. ROETHLER[1], K. COHEN-KASHI[1], L. BITAN[1], H. W. GABEL[2], I. SPIEGEL[1];
[1]Dept. of Brain Sci., Weizmann Inst. of Sci., Rehovot, Israel; [2]Dept. of Neurosci., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Experience-regulated genomics enhancers are thought to control the function and plasticity of neural circuits — e.g., during learning — by regulating the experience-dependent transcription of genes that, in turn, modulate specific cells and synapses. However, it remains largely unknown which enhancers control the experience-induced transcription of specific genes, and thus, the cellular and circuit functions of such experience-regulated enhancers could not be tested. To start dissecting the neurobiological functions of experience-induced enhancers *in vivo* in the adult brain, here we focus on the *Igf1* gene which encodes the secreted Insulin-like Growth Factor 1 (IGF1) and which is expressed and experience-induced in the adult visual cortex selectively in disinhibitory VIP interneurons (INs). Using cell-type-specific Chip-Seq and newly generated mouse alleles, we identify two experience-induced enhancers upstream of the *Igf1* locus and demonstrate that these genomic sites selectively control the experience-induced transcription of *Igf1* but not its basal transcription (i.e., non-experience-regulated). Furthermore, when we use patch-clamp electrophysiology in acute brain slices and intersectional genetics to knock out these enhancers acutely and selectively in VIP INs in the adult visual cortex, we find that these enhancers control the E/I-ratio in VIP INs by promoting inhibitory inputs onto VIP INs in an experience-dependent and a cell-autonomous manner. Finally, by performing calcium imaging in the visual cortex of awake behaving mice, we demonstrate that these enhancers control the activity of VIP INs and control visual processing *in vivo*. Taken together, these experiments demonstrate that enhancer-mediated experience-induced transcription of a single gene can control E/I-ratio in single neurons and that this is necessary for proper visual processing in the adult visual cortex. Therefore, we conclude that the enhancer-mediated experience-induced transcription dynamically maintains and fine-tunes information processing in adult neural circuits.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Program #/Poster #: 611.01

Topic: B.09. Glial Mechanisms

Support: F99NS124179
R01NS120746
R01HL104101

Title: Astrocyte TrkB.T1 activation by BDNF elicits morphological responses

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Abstract: Astrocytes are crucial support cells within the CNS, known for their roles in ionic and transmitter homeostasis, injury response, and BBB maintenance. Astrocytes undergo postnatal maturation alongside neurons, indicated by changes in astrocytic gene expression, cellular function, and development of a complex morphological phenotype. Few mechanisms have been described that link the coordinated refinement of neurons and astrocytes across development. Our recent publication describes an astrocyte-specific mechanism of morphological maturation that implicates brain-derived neurotrophic factor (BDNF) signaling through the astrocyte truncated TrkB receptor (TrkB.T1) as critical for astrocyte development. Others have found that TrkB.T1 associates with the RhoGTPase inhibitor RhoGDIα. Our preliminary findings in acutely isolated astrocytes demonstrates RhoGTPase inhibition after 1 hour of BDNF treatment, suggesting that BDNF/TrkB.T1 signaling may modulate actin dynamics at particular timescales. We used time-lapse confocal microscopy to evaluate mouse WT and TrkB.T1 KO primary astrocyte responses to BDNF over a 12 hour treatment period and identified BDNF-dependent morphological changes in actin surface area. Our ongoing work seeks to elucidate the RhoGTPase signaling mechanisms that mediate TrkB.T1/BDNF induced morphological changes.

Disclosures: R.D. Hernandez: None. **S.G. Watsen:** None. **M.L. Olsen:** None.

Poster

611. Astrocytic Mechanisms of CNS Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 611.02

Topic: B.09. Glial Mechanisms

Support: Korea Brain Research Institute funded by the Ministry of Science and ICT (22-BR-01-02)

Title: Molecular mechanisms underlying endocytic BDNF secretion in astrocytes

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Abstract: Brain-derived neurotrophic factor (BDNF) regulates diverse brain functions via TrkB receptor signaling. Astrocytes are known to recycle the neuronal BDNF proteins by internalizing extracellular BDNF proteins via TrkB receptor-mediated endocytosis and re-secreting endocytosed BDNF upon stimulation. However, the molecular mechanism underlying such astrocytic BDNF recycling remains unrecognized. To explore the mechanisms for astrocytic BDNF recycling, we monitored the uptake, transport, and secretion of BDNF from cultured cortical astrocytes by using quantum dot (QD)-conjugated mature BDNF (QD-BDNF) as a proxy

for the extracellular BDNF protein. Assessing subcellular localization of intracellular QD-BDNF with diverse vesicular markers we revealed the high colocalization ratio between endocytic BDNF and vesicle-associated membrane protein 3 (Vamp3) in astrocytes. This suggests that Vamp3-dependent signaling is involved in endocytic BDNF processing in astrocytes. ATP stimulation of the QD-BDNF-containing astrocytes triggered either the antero- or retrograde transport of QD-BDNF containing vesicles, but downregulation of Vamp3 expression by siRNA transfection did not affect uptake or transport of QD-BDNF. However, ATP-induced secretion of endocytic BDNF (QD-BDNF) and astrocyte-synthesized BDNF (BDNF-EGFP) was sensitive to Vamp3 expression, indicating that astrocytic Vamp3 is selectively required for exocytosis of endocytic BDNF or astrocyte-synthesized BDNF. Additional tests for QD-BDNF localization showed that endocytic QD-BDNF are significantly included in secretory granules containing BDNF-EGFP, but such colocalization between QD-BDNF and BDNF-EGFP was independent of the Vamp3 expression. Collectively, these results propose secretion of endocytic BDNF could share the molecular mechanisms with that of astrocyte-synthesized BDNF, and Vamp3 plays a central role in controlling regulatory secretory pathways to release both types of BDNF proteins from astrocytes.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

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Title: Distribution and functional significance of cerebellar glycogen in the mouse

Authors: S. AKTHER[1,2], A. B. LEE[1], A. KONNO[3,4], A. ASIMINAS[1], M. VITTANI[1], T. MISHIMA[1], H. HIRAI[3,4], C. F. MEEHAN[1], J. DURAN[5,6,7], J. GUINOVART[5], H. ASHIDA[8], T. MORITA[9], O. BABA[9], R. SHIGEMOTO[10], M. NEDERGAARD[1,11], *H. HIRASE[1,2,11];
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Abstract: The mammalian brain stores glucose, the main energy substrate from the blood circulation, in the form of glycogen. In the rodent brain, the cerebellum stores relatively large amounts of glycogen, yet the cellular and subcellular distribution of glycogen has not been described in detail. Using monoclonal antibodies against glycogen, we investigated the glycogen distribution in the mouse cerebellar cortex. We found a dominant presence of glycogen in molecular layer Bergmann glia (BG) processes. Additionally, glycogen was observed in Purkinje cells (PCs), the principal neurons of the cerebellar cortex. To address the functional significance of cerebellar glycogen, we examined behavioral phenotypes in mice that lack glycogen synthase 1 (Gys1) in cerebellar astrocytes (which include BG) or PCs using a Gys-1 floxed mouse line. Gys1 deficiency in PCs (by L7-Cre transgenic mouse or AAV-L7-Cre) or AAV-GFAP-Cre-infected cells alone did not result in a notable phenotype, however, Gys1 deficiency in both cell types induced ataxia. Histological examination showed that the dual cell-type gys1 deficiency ablated PCs. Our observations elucidate a critical involvement of glycogen metabolism for the normal operation of the cerebellum.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

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Healthy Brains Healthy Lives
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Title: Calcium events in visual cortex astrocytes desynchronize with development

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Abstract: Cortical astrocytes signal and communicate via intracellular Ca^{2+} transients. This Ca^{2+} activity have also been shown to be involved in controlling synaptic plasticity at nearby synapses. We showed that astrocyte spontaneous Ca^{2+} activity becomes less synchronous within individual cells over the development from postnatal days (P) 3 - 30 in C57BL/6 mice. This is correlated with maturation of astrocyte electrophysiology properties, gap-junction development, and morphology maturation.

Acute slices were pre-incubated in 1 μM sulforhodamine 101 for 5 minutes. This dye was selectively taken up by astrocytes and allowed for targeted whole-cell patching under 2-photon microscopy (2p). Cells in layer 5 visual cortex were patched and voltage clamped at -80 mV, and 500 ms voltage steps from -60 mV to +160 mV were applied to generate current-voltage (IV) curves. This revealed at least two response types: a steady, time-invariant conductance, and a slowly activating component. The latter diminished with age (Spearman's rho = -0.44, $p < 0.05$, $n = 22$). In addition, the average V_m was -83 ± 0.3 mV and average R_{input} was 29 ± 1.3 MΩ ($n = 196$). Across ages P3 - 30, V_m increased (Pearson's $r = 0.438$, $p < 0.01$) and R_{input} decreased ($r = -0.159$, $p < 0.05$).
To investigate the development of gap-junction coupling in these cells, astrocytes were patched and filled with biocytin for 15 minutes. Slices were fixed in paraformaldehyde and stained with Alexa Fluor-conjugated streptavidin. Cell counts were done for biocytin positive astrocytes as a quantification of amount of gap-junction coupling. We found that the number of coupled cells increased with age starting around P7 ($r = 0.73$, $p < 0.05$, $n = 24$).
Manual 3D reconstructions from 2p imaging of Alexa Fluor 488 loading or from confocal imaging of biocytin staining were done using the software Neuromantic. Analysis revealed increased branch density, especially closer towards the soma, over development. We also saw reduced maximal extent of reach of astrocyte branches after P20 based on Sholl analysis.
Finally, we visualized spontaneous Ca^{2+} transients with 2p of Fluo-5F (200 μM) or AAV-GCaMP6f. By manually selecting individual regions of interest and running a correlation analysis on every region, we found that events within cells decorrelated with age ($r = -0.57$, $p < 0.01$, $n = 20$). Automatic Ca^{2+} event detection also showed that they became more frequent ($r = 0.581$, $p < 0.01$) and shorter in duration with age ($r = -0.632$, $p < 0.01$).
As astrocytes mature, spontaneous Ca^{2+} activity changes from slow synchronous cell-wide waves to quick local transients. This decorrelation may enable localized astrocytic control of synaptic plasticity.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

Support: NIH NINDS R21 NS116664

Title: Sensory experience stimulates neuron-astrocyte Sonic Hedgehog signaling

Authors: *A. LE[1], S. HILL[3], M. FU[1], A. GARCIA[1,2];
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Abstract: Astrocytes, the most abundant subtype of glial cells in the central nervous system, closely associate with neuronal synapses through their dynamic distal processes. While the role of astrocytes in synapse formation and function is well characterized, their role in synaptic plasticity is less well understood. Growing evidence suggests astrocytes can facilitate experience-dependent neuronal plasticity, however the mechanism by which they accomplish this is poorly understood. The Sonic hedgehog (Shh) signaling pathway is a compelling candidate for mediating this bidirectional communication. Although best known for patterning the nervous system in development, previous work in the lab has demonstrated that Shh persists into adulthood, expressed by neurons localized to layer V and transduced by a subpopulation of layer IV and V astrocytes. We showed that Shh signaling is required to establish synaptic organization during postnatal development and promotes structural plasticity of synapses. In this study, we examined whether Shh signaling can be regulated by activity. We housed mice in an enriched environment to promote robust whisking and somatosensory activity. We found that enriched sensory experience stimulated an increase in Shh signaling in the somatosensory, but not motor and visual cortex, suggesting that experience-dependent Shh signaling occurs in a modality-specific manner. To confirm whether neuronal activity alone can stimulate Shh activity, we activated neurons using a chemogenetic approach and observed increased Shh signaling. Next, to identify potential activity-dependent genes regulated by Shh signaling, we performed bulk RNA sequencing to identify genes enriched in Shh-transducing astrocytes. We identified SPARC and Hevin - astrocyte-secreted matricellular proteins that regulate synaptic formation, function and plasticity - as selectively enriched in deep layer cortical astrocytes, where astrocytic Shh activity is most pronounced, compared with the total cortical astrocyte population. Disruption of Shh signaling in astrocytes reduced *Sparc* and *Hevin*, identifying these genes as Shh-dependent. Ongoing work will determine whether SPARC and Hevin can be regulated *in vivo* by enriched experience. In summary, our work revealed a novel activity-dependent feature of neuron-astrocyte Shh signaling, deepening our understanding of neuron-astrocyte bidirectional communication in experience-dependent plasticity.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Program #/Poster #: 611.06

Topic: B.09. Glial Mechanisms

Title: Cellular differences in ceramide homeostasis affects energy metabolism in iPSC derived motor neurons, astrocytes, and microglia

Authors: *J. MCINNIS, D. SOOD, M. GONCALVES, L. GUO, B. ZHANG, J. DODGE; Sanofi-Aventis Pharmaceuticals, Cambridge, MA

Abstract: Cellular differences in ceramide homeostasis affects energy metabolism in iPSC derived motor neurons, astrocytes, and microglia John McInnis[1], Disha Sood[1], Mariana Goncalves[2], Lilu Guo[2], Bailin Zhang[2], James Dodge[11]Sanofi, Rare and Neurologic Disease, 350 Water Street, Cambridge, MA 02141[2]Sanofi, Precision Medicine and Computational Biology, 350 Water Street, Cambridge, MA 02141

Lipid homeostasis is essential for the normal development, maturation, health, and function of the CNS. Ceramides are sphingolipids that play essential roles in lipid rafts, membrane curvature, mitochondrial function, and cell death. Ceramide synthases (CerS 1-6) are part of the *de novo* and salvage ceramide synthesis pathways, with each CerS producing ceramides of specific carbon chain lengths. Genetic, transcriptional, and biochemical evidence indicates that aberrations in ceramide synthesis contribute to neuronal dysfunction in neurodegenerative diseases. Understanding of how ceramide synthesis and catabolism is regulated in neurons and glia is very limited. Here, we utilized human induced pluripotent stem cell-derived astrocytes, microglia, and motor neurons, to characterize ceramide regulation in each of these CNS cell types. Glia and motor neurons had significantly different ceramide profiles, expression of ceramide metabolic enzymes, levels of ceramide synthase activity and response to ceramide synthase inhibition. Interestingly, ceramide synthase activity was more predictive of the ceramide profiles and turnover than CerS gene expression, pointing to a possible role for post-translational modifications. In comparison to motor neurons, glia had both more rapid incorporation of labelled precursors into ceramides and greater reduction of ceramides following synthesis inhibition. This suggests that cell-type specific differences in ceramide regulation could account for the sensitivity of neurons to cell death in neurodegenerative disease. Additionally, based on the known function of ceramide in regulating mitochondrial function, we showed cell-type specific differences in baseline mitochondrial function, as well as mitochondrial responses to ceramide synthesis pathway inhibitors, by Seahorse metabolic assays. The results of our experiments are critical for our understanding of cell-type specific metabolic regulation and sensitivity of cells to metabolic perturbations, with possible implications for drug targeting.

Disclosures: **J. McInnis:** A. Employment/Salary (full or part-time):; Sanofi. **D. Sood:** A. Employment/Salary (full or part-time):; Sanofi. **M. Goncalves:** A. Employment/Salary (full or part-time):; Sanofi. **L. Guo:** A. Employment/Salary (full or part-time):; Sanofi. **B. Zhang:** A. Employment/Salary (full or part-time):; Sanofi. **J. Dodge:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

Support: This research is supported by the intramural research program of National Institutes of Health. Project number 1ZIANS003137

Title: Genetic dissection of cholesterol synthesis pathway in the Drosophila brain

Authors: *A. GRIGSBY-BROWN[1], J. YIN[1], C.-I. J. MA[2], J. SHORT[1], J. A. BRILL[2], Q. YUAN[1];
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Abstract: As a major component of eukaryotic plasma membranes, cholesterol influences membrane permeability and fluidity, and supports cellular structures and transport. In the mammalian central nervous system (CNS), cholesterol is enriched within myelin sheath, regulates neuronal signal transduction, acts as precursors to many steroid hormones, and is required for synapse and dendrite formation during development. The impairment of cholesterol metabolism and transport has been linked to neurodegenerative disorders, including Alzheimer's Disease and Parkinson's Disease. Given the critical functions of cholesterol in the nervous system, its content in mammalian brains is tightly controlled by *de novo* synthesis and excretion and involves trafficking between neuron and glia. *Drosophila melanogaster* emerges as a valuable model system for understanding molecular mechanisms underlying neuron-glia lipid trafficking. However, *Drosophila* has been shown to be sterol auxotroph and requires dietary cholesterol input to produce essential hormones. Whether cholesterol synthesis exists in the fly brain and how it is regulated remains unknown. To address these questions, we performed genetics and imaging experiments. Using fluorescent lipid indicators, our preliminary results suggest that similar as the case in the mammalian brain, the fly blood-brain-barrier (BBB) prevents entry of dietary cholesterol into the CNS. In addition, the brain content of cholesterol remains steady when it is eliminated from the diet, suggesting possible *de novo* synthesis of cholesterol in the fly CNS. This hypothesis is further supported by our RNA-seq analyses and genetic studies, which reveal the presence of fly homologues of a complete cholesterol synthesis machinery in the larval brain transcriptome, as well as candidate genes that regulate brain cholesterol contents. Our study offers molecular insights into the unique mechanisms controlling neuron-glia lipid trafficking and cholesterol homeostasis in the *Drosophila* brain, which will help us better understand cholesterol related neurodevelopmental regulations and neurodegenerative disorders.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

Support: NS034007
NS122316

Title: Astrocytes synthesize and transfer protein synthesis regulators into neurons

Authors: *W. J. LIU[1], M. M. OLIVEIRA[2], E. KLANN[2];
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Abstract: Neuronal protein synthesis is indispensable for long-lasting synaptic plasticity and long-term memory. Although translational regulation in plasticity and its dysregulation in disease has been well characterized in neurons, it is unknown whether this process is cell autonomous, or whether other cell types are involved. Because astrocytes are known to secrete a wide range of trophic, supporting factors that promote neuronal survival and function, we first sought to determine whether astrocytes can regulate neuronal translation. Using primary mouse astrocyte cultures, we labeled newly synthesized proteins by spiking into the medium puromycin, an aminonucleoside that enters the ribosomal A site to transfer to a growing peptide, leading to premature chain termination and release. After a washout of excess puromycin, we collected the astrocyte-conditioned medium (ACM) and used this ACM to treat primary mouse cortical neuron cultures. Using proximity-ligation assays (PLA) for puromycin and GADD34, a scaffold for protein phosphatase 1 that promotes the dephosphorylation of the translation intitiation factor eIF2α, we detected PLA signal in neurons treated with puromycin-labeled ACM. This result suggests that astrocytes synthesized new GADD34 proteins that were then transferred into neurons, hinting at a potential non-cell-autonomous mechanism for regulation of neuronal protein synthesis. In addition, treatment of astrocytes with brain-derived neurotrophic factor (BDNF) significantly increased the number of puromycin-GADD34 PLA puncta ($p<0.01$), suggesting that astrocytic regulation of neuronal protein synthesis is also dependent upon neuronal activity. Taken together, these findings suggest that astrocytes can regulate neuronal protein synthesis in a physiolgical non-cell autonomous manner that may be important for long-lasting synaptic plasticity and memory. Moreover, disruption of this astrocyte-neuron communcation might underlie impaired protein synthesis observed in neurodegenerative diseases such as Alzheimer's disease.This work was supported by NIH grants NS034007 and NS122316 (E.K.).

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Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

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Title: Astrocytic but not microglial phagocytosis is mediated by the phagocytic receptor Adgrb1/Bai1

Authors: *F. SHIU[1], J. C. WONG[2], S. A. SLOAN[3], A. ESCAYG[3];
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Abstract: The phagocytic G protein-coupled receptor BAI1/ADGRB1 has been shown to be associated with autism spectrum disorder. We recently demonstrated that *Adgrb1*$^{-/-}$ mice exhibit impairments in social behavior and increased seizure susceptibility. Interestingly, mice lacking other phagocyte receptors or pathway components show behavioral deficits that overlap with *Adgrb1*$^{-/-}$ mice. While knockout of *Adgrb1* results in higher levels of apoptotic cells in skeletal muscle and colonic epithelial cells, the role of the receptor in phagocytosis is not well studied in the brain. Expression analysis has revealed that *Adgrb1* is highly expressed in astrocytes but not in microglia. Thus, we hypothesized that astrocytes lacking Adgrb1 would have reduced phagocytic ability, which in turn might contribute to the behavioral deficits observed in the *Adgrb1*$^{-/-}$ mice. As Adgrb1 has the highest expression at postnatal 7 (P7) in rodents, we compared hippocampal astrocytes and levels of apoptotic cells and synapses in P7 *Adgrb1*$^{-/-}$ mice and WT littermates. We observed greater GFAP immunofluorescence (astrocyte marker) and a higher number of cleaved caspase 3 positive cells (apoptosis marker) in the knockout mice. Cultured astrocytes from *Adgrb1*$^{-/-}$ mice exhibited less phagocytic capacity when compared to similarly prepared astrocytes from WT littermates. In contrast, no significant differences of phagocytic capacity of cultured microglia from *Adgrb1*$^{-/-}$ mice and WT littermates were observed. Furthermore, in P7 mice, a significantly higher volume of synapsin 1 (a pre-synaptic marker) was observed in *Adgrb1*$^{-/-}$ mice when compared to WT littermates, while the volume of the post-synaptic marker (homer 1) was comparable between the two genotypes. Synapse staining at P90 revealed a significantly higher volume of both pre- and post-synaptic markers in *Adgrb1*$^{-/-}$ mutants. These results demonstrate that *Adgrb1*$^{-/-}$ mutants have reduced astrocyte mediated phagocytosis which may result in higher levels of uncleared apoptotic cells and unpruned synapses.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

Support: NIH Grant RO1NS116059

Title: Activation of Gq-DREADD in astrocytes by micromolar clozapine-N-oxide/clozapine is lethal to neurons and astrocytes through impairment of astrocyte potassium conductance in mice hippocampus

Authors: *Y. LUO[1], A. LI[2], L. TRANK[2], S. ATEN[2], K. GOGIN[2], M. WITT[1], X. LIU[3], M. ZHOU[4], Y. DU[4];
[1]Ohio State Univ., [3]Wexner Med. Ctr., [4]the Ohio State Univ., [2]Ohio State Univ., Columbus, OH

Abstract: Chemogenetics is increasingly used in studies of astrocyte physiology and pathology. The clozapine N-oxide (CNO) doses from 1 μM - 1 mM and a perfusion duration of 35 - 60 min are commonly used to activate astrocyte-expressing Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). While clozapine, a metabolite of CNO, appears to be the actual agonist for DREADDs at a higher affinity and potency. However, little is known about the direct impact of different doses of CNO/clozapine on the function and viability of Gq-DREADD expressing astrocytes. We used animal genetics to introduce Gq-DREADD to astrocytes, validated their functional expressions by calcium imaging, and used electrophysiology and TO-PRO-3-I staining to assess the effective CNO/clozapine doses that could be safely used for astrocyte physiology studies. Results show that activation of Gq-DREADD in astrocytes induces intracellular calcium increase in a dose-dependent manner and the calcium raise sustains even after 10 min of recovery. What's more, although nanomolar CNO/clozapine does not alter the basic properties of Gq-DREADD-expressing astrocytes *in situ*, micromolar CNO/clozapine dose-dependently depolarizes the astrocyte membrane potential and inhibits passive K^+ conductance, indicating an excessive elevation of intracellular Ca^{2+} in Gq-DREADD expressing astrocytes has a detrimental impact on the physiological properties of astrocytes. To explore any collateral impact on neurons, TO-PRO-3-I staining was used to assess cell viability after a 10 min activation of astrocyte-expressing Gq-DREADD by micromolar CNO/clozapine, and the results showed a massive neuronal death in hippocampal pyramidal and striatum radiatum regions. Since a longer than 10 min CNO/clozapine application is commonly used, more severe damage to astrocytes and neurons would be expected. Therefore, we conclude that when coming to interpret changes in neuronal circuit and animal behaviors induced by a moderate long exposure of Gq-DREADD expressing astrocytes to micromolar CNO/clozapine, it is necessary to consider the co-existing viability reduction of astrocytes and collateral impact. And we would recommend researchers use nanomolar clozapine to safely induce calcium elevation in Gq-DERADD expressing astrocytes.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Support: SSTF-BA1502-13
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Title: Prefrontal astrocytes control mouse dominance behavior and social hierarchy by regulating synaptic excitatory and inhibitory balance

Authors: *K. NOH[1], B. LEE[1], W.-H. CHO[1], K. PARK[1], M. HWANG[1], Y. CHO[2], Y. KIM[3], B.-E. YOON[3], S.-Y. CHOI[1], H. PARK[1], **S. LEE**[1];
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Abstract: Social hierarchy in animals establishes as an outcome of individual social behavior, such as dominance behavior during long-term interactions. Astrocytes are implicated in optimizing the balance between excitatory and inhibitory (E/I) neuronal activities that are crucial for social behavior. However, the contribution of astrocytes to dominance behavior is unknown. Here, we aimed to decipher the role of dorsomedial prefrontal cortex (dmPFC) astrocytes in mouse dominance behavior. *In vivo* fiber photometry and two-photon microscopy showed that dmPFC astrocyte calcium activities are correlated with mouse dominance behavior. Additionally, optogenetic stimulation or pharmacological astrocyte ablation bidirectionally controlled mouse dominance behavior and social rank. Dominant and subordinate mice presented distinct prefrontal synaptic E/I balance, regulated by astrocytes activity. As a mechanism, dmPFC astrocytes controlled synaptic E/I balance by presynaptically enhancing excitatory synaptic transmission and postsynaptically reducing inhibitory synaptic transmission simultaneously via astrocyte-derived glutamate and ATP, respectively. Our findings reveal a novel role for dmPFC astrocytes in the regulation of mouse social hierarchy and provide new perspective on brain functioning mechanisms of dominance behavior based on neuron-astrocyte communication.

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Poster

611. Astrocytic Mechanisms of CNS Function

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Support: KHIDI grant HU20C0206

Title: Lysosomal stress-induced TFEB activation requires zinc release from lysosomes

Authors: *B.-R. SEO[1], J. CHOI[1], Y. YOON[2], J.-Y. KOH[1,3];
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Abstract: Transcription factor EB (TFEB) is the master switch for the activation of the CLEAR network genes that encode proteins involved in the catabolism of waste proteins and organelles. As such, TFEB activation and upregulation of the CLEAR network proteins may be an important reaction to lysosomal stress. Several reports showed that calcium release from stressed or dysfunctional lysosomes may play a key role in the activation of TFEB and its translocation to nuclei. Since we previously showed key roles of zinc in the maintenance of lysosomal function, we examined whether lysosomal zinc also plays a role in TFEB activation under lysosomal stress in cultured cortical astrocytes. To induce lysosomal stress in cultured cortical astrocytes, we used BafA1, a potent and selective inhibitor of the lysosomal proton pump, vATPase. Treatment with 100 nM BafA1 increased lysosomal pH and caused reduction in p62 degradation as previously reported. After 1 hr of BafA1 treatment, TFEB was found to be translocated from cytosol to nuclei. Concomitantly, staining to BafA1 treated astrocytes with fluorescence dyes for zinc showed that while zinc fluorescence in lysosomes decreased, zinc levels in the cytosol increased. In contrast, fluo-8 straining did not detect changes in calcium fluorescence. Consistently, addition of zinc chelator TPEN completely blocked TFEB translocation upon BafA1 treatment/lysosomal stress. Furthermore, increasing cytosolic zinc levels with Zn-Clioquinol was sufficient in inducing TFEB translocation in naïve astrocytes. It has been reported that TRPML1 channels may be the main route for release of zinc and calcium from lysosomes. Indicating that TRPML1 channels may mediate this zinc movement, an inhibitor of TRPML1 channel ML-SI3 blocked it. Conversely, a TRPML1 activator ML-SA1 increased cytosolic zinc levels and activated TFEB translocation in a TPEN-blockable fashion. Again, we failed to find an increase in calcium signals upon TRPML1 activation by ML-SA1. Our result suggests that under lysosomal stress, release of zinc rather than calcium from lysosomes to the cytosol via TRPML1 channels may be required to activate TFEB. Hence lysosomal zinc may act as a key ionic mediator to deliver lysosomal stress signals via TFEB to the nucleus.

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Poster

611. Astrocytic Mechanisms of CNS Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 611.13

Topic: B.09. Glial Mechanisms

Support: NIH-AG027956

Title: Connexin 43 gap junctions as novel targets/mediators of estrogen-induced protection of cortical astrocytes

Authors: *V. KRISHNAMOORTHY, S. KIM, H. LAPORTE, M. SINGH;
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Abstract: Connexin 43 (Cx43) is a protein that is abundantly expressed in astrocytes and plays a role in influencing cell viability and intercellular communication. Specifically, Cx43 can exist in two distinct conformations, either as hemichannels or gap junctions. Previous studies have demonstrated that gonadal steroid hormones, including estrogens, may influence Cx43 function in peripheral tissue, but this mechanism of estrogen action in the brain has not been established. As such, we explored the effects of estrogens on Cx43 function and permeability in cerebral cortical astrocytes and hypothesized that Cx43 is a novel downstream mediator of estrogen-induced cytoprotection. To test our hypothesis, cultures of cerebral cortical astrocytes derived from neonatal female C57/Bl6 mice were used to determine whether 17β-estradiol (E2) elicited changes in Cx43 mRNA expression and Cx43 permeability. Cultures were treated with 100 nM E2, or estrogenic metabolite of dihydrostesterone (DHT), 5-α-androstane-3,17-β-diol (3βdiol), in the presence (or absence) of a metabolic/oxidative insult, iodoacetic acid (IAA). Additionally, to determine whether Cx43 hemichannels or gap junctions mediated the protective effects of estrogens, pharmacological agents for a hemichannel blocker (Gap19) and a gap junction blocker (Gap26) were used. A Pannexin 1 blocker (10Panx) was also used to rule out the potential involvement of Pannexin 1 channels. Following treatment, cell viability was assessed using an MTT viability assay and changes in mRNA expression were evaluated using RT-qPCR. We also used dye uptake assays, using ethidium bromide (EtBr) or lucifer yellow dye uptake assays, to determine whether estrogens influenced hemichannel or gap junction permeability, respectively. Results from this study indicate that E2 and 3βdiol treatment led to an increase in the levels of Gap26 mRNA, a negative regulator of Cx43 function/permeability, but had no effect on Cx43 expression. Consistent with the effects on Gap26 expression, E2 and 3βdiol treatment each led to the inhibition in Cx43 gap junction permeability, an effect that correlated with an increase in cytoprotection. No effects were observed on Cx43 hemichannel permeability. In conclusion, data from this study suggests that E2 and 3βdiol promote cytoprotection in cortical astrocytes by targeting the function of Cx43 gap junction proteins, and identify Cx43 gap junctions as a potential druggable target for the treatment of brain disorders where astrocyte viability/function is compromised.

Disclosures: **V. Krishnamoorthy:** None. **S. Kim:** None. **H. LaPorte:** None. **M. Singh:** None.

Poster

611. Astrocytic Mechanisms of CNS Function

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Topic: B.09. Glial Mechanisms

Support: National Institutes of Health grant K99/R00AG048222
Leon Levy Foundation Fellowship in Neuroscience
National Science Foundation Graduate Research Fellowship #191335-02

Title: Astrocyte metabotropic receptors influence spatial memory in a sex-specific manner

Authors: S. M. MEADOWS[1,2,3], F. PALAGUACHI[1,2], A. LICHT-MURAVA[1,2], T. S. ZIMMER[1,2], D. BARNETT[1,2,3], A. L. ORR[1,2], A. G. ORR[1,2];
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Abstract: Astrocytes are abundant cells in the central nervous system that regulate and support diverse neural functions. Activation of astrocytic G protein-coupled receptors (GPCRs) is a key mechanism through which astrocytes sense and regulate neural activities and behavior. It is well-established that the effects of astrocytic GPCRs are highly context-dependent and can vary with brain region, age, and other factors. However, it is not known if biological sex plays a significant role in how astrocytic receptors modulate behavior. Here, we tested whether astrocytic GPCRs regulate learning and memory in a sex-specific manner using four complementary *in vivo* manipulations. We first utilized CRISPR-Cas9 to inducibly knock down astrocytic metabotropic glutamate receptor 3 (mGluR3) in the hippocampus of adult mice. mGluR3 is a $G_{i/o}$-coupled receptor that serves as a central astrocytic sensor of glutamate, but its effects on brain function are not clear. We found that astrocyte-targeted knockdown of mGluR3 altered spatial memory in both sexes, but the effects differed in males and females. Specifically, mGluR3 knockdown impaired memory in females, but improved memory in males, suggesting that astrocytic mGluR3 regulates memory in a sexually dimorphic manner. To further test if changes in mGluR3 expression cause bidirectional effects on memory, we used Cre-dependent viral vectors to enhance astrocytic mGluR3 levels. Increasing mGluR3 improved memory in females but not males, indicating that the sex-specific effects of mGluR3 were bidirectional and not unique to the knockdown approach. We next tested if the sex-specific effects occurred with other types of astrocytic GPCRs. For these experiments, we used *in vivo* chemogenetic stimulation to selectively activate $G_{i/o}$- or G_s-coupled receptor signaling in hippocampal astrocytes during learning. In congruence with the effects of mGluR3 manipulation, activating astrocytic $G_{i/o}$- or G_s-coupled receptors modulated spatial memory in a bidirectional and sex-specific manner, suggesting that signaling by various astrocytic GPCRs is sufficient to induce sex-specific effects on memory. Additional methods were used to address potential mechanisms mediating these effects. Together, our findings reveal that astrocytic mGluR3 and other GPCRs regulate hippocampal function in a highly sex-specific manner, and alterations in astrocytic receptor signaling might contribute to sex differences in health and disease.

Disclosures: S.M. Meadows: None. **F. Palaguachi:** None. **A. Licht-Murava:** None. **T.S. Zimmer:** None. **D. Barnett:** None. **A.L. Orr:** None. **A.G. Orr:** None.

Poster

611. Astrocytic Mechanisms of CNS Function

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Program #/Poster #: 611.15

Topic: B.09. Glial Mechanisms

Support: Wallace Coulter Foundation

Title: Novel Nonlinear Approach to Characterizing Astrocytic Neurovascular Coupling with Optogenetics and Biophysical Modeling

Authors: L. FERNANDEZ[1], **A. SUAREZ**[2], *J. RIERA[1];
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Abstract: Astrocyte-mediated neurovascular coupling is a series of biomechanical pathways by which resource delivery to neural tissue is regionally optimized. These pathways, which depend on astrocytic calcium signaling, have been actively charted in past decades but quantification has proven controversial when components are considered as making additive contributions to hemodynamic change in blood vessels. We hypothesize that neurovascular coupling is instead enacted through a dynamic, nonlinear fashion. To evaluate our hypothesis, we utilized a tetracycline based ChR2-EYFP transgenic mouse model which was optogenetically stimulated through light sensitive ion channels expressed in cortical astrocytes. Hemodynamic change was measured via Laser Doppler Flowmetry modality and elicited through a calcium signaling generated by 470nm blue light stimulation. Stimulated regions demonstrated a 20% sustained increase in localized perfusion. We also set out to evaluate individual pathway components through pharmacological inhibition. We proposed that a population of Group IIA secretory phospholipase A2 (sPLA2) contributes alongside Group IV cytosolic PLA2 (cPLA2) to enact hemodynamic change. We hypothesize that PLA2-dependent pathways would be mediated through use of Group IIA inhibitor Varespladib and Group IV inhibitor MAFP. After blocking cPLA2 mediated pathways we found a significant reduction in CBF during stimulation, suggesting an active role in vasodilation. However, inhibition of sPLA2 induced oscillatory behavior in the CBF baseline following drug application, implying a pathway contribution towards maintaining vascular tone. Lastly, we are developing a computational biophysical model of the hemodynamic response encompassing all known calcium wave to SMC hemodynamic mechanisms. By adopting a prior theoretical model of optogenetically-induced calcium activity from our lab, we began a comprehensive literature review to determine model equations, state variables, and physiological parameters. Results from the model show differences in the spatiotemporal features of the response depending on the characteristic dynamics of each bio-signaling pathway. These results agree with experimental data from transgenic mice where an observed rapid increase in the signal is followed by a slow recovery towards baseline.
Our findings demonstrate a significant contribution towards resource delivery by astrocytic calcium-dependent pathways. We believe our findings provide a new approach by which future studies may continue to evaluate neurovascular coupling and characterize this phenomenon within the greater context of the nervous system.

Disclosures: L. Fernandez: None. **A. Suarez:** A. Employment/Salary (full or part-time):; Florida International University. **J. Riera:** 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.; Florida International University.

Poster

611. Astrocytic Mechanisms of CNS Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 611.16

Topic: B.09. Glial Mechanisms

Support: CIHR

Title: Astrocyte Ca^{2+} Dynamics in Distinct Functional Compartments Differ in Response to Vasoconstriction

Authors: *K. A. GORZO, G. R. GORDON;
Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Located at a central position within the neurovascular unit, each astrocyte is tasked with integrating information from both neurons and the microvasculature to coordinate brain activity and metabolism. This is accomplished, in part, through calcium (Ca^{2+}) transients inside distinct functional compartments of the astrocyte. It is currently unknown how constriction and dilation of blood vessels alters different types of microdomain Ca^{2+} transients within the astrocyte, and thus influences downstream cellular signalling. To investigate this, we used 2-photon microscopy to visualize genetically encoded Ca^{2+} indicators (GECIs) targeted either to the plasma membrane or the cytosol of the astrocyte in response to vasoconstriction. Our data demonstrates that in response to vasoconstriction, plasma membrane Ca^{2+} transients in the astrocyte arbour increase in frequency, amplitude, and area, whereas cytosolic Ca^{2+} transients decrease in frequency, amplitude, and area. Additionally, the co-application of mitochondrial inhibitors cyclosporin A and rotenone prevented the observed decreased in frequency, amplitude, and area of cytosolic Ca2+ transients in response to vasoconstriction. These distinctly localized and opposing changes observed in astrocyte arbour Ca^{2+} may represent separate cellular mechanisms responding to vasoconstriction. Understanding these processes will likely uncover a new means through which astrocytes sense changes in the brain micro-environment and regulate gliotransmission accordingly.

Disclosures: K.A. Gorzo: None. **G.R. Gordon:** None.

Poster

611. Astrocytic Mechanisms of CNS Function

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Program #/Poster #: 611.17

Topic: B.09. Glial Mechanisms

Support: NS113499
NS104478
NS100796
NS127819

Title: Neuronal activity drives pathway-specific depolarization of peripheral astrocyte processes

Authors: *M. ARMBRUSTER[1], C. G. DULLA[2];
[1]Neurosci., Tufts Univ., Boston, MA; [2]Tufts Univ. Sch. of Med., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Astrocytes are glial cells that interact with neuronal synapses via their distal processes, where they remove glutamate and potassium (K+) from the extracellular space following neuronal activity. Astrocyte clearance of both glutamate and K+ is voltage dependent, but astrocyte membrane potential (Vm) is thought to be largely invariant. As a result, these voltage dependencies have not been considered relevant to astrocyte function. Using genetically encoded voltage indicators to enable the measurement of Vm at peripheral astrocyte processes (PAPs) in mice, we report large, rapid, focal and pathway-specific depolarizations in PAPs during neuronal activity. These activity-dependent astrocyte depolarizations are driven by action potential-mediated presynaptic K+ efflux and electrogenic glutamate transporters. We find that PAP depolarization inhibits astrocyte glutamate clearance during neuronal activity, enhancing neuronal activation by glutamate. This represents a novel class of subcellular astrocyte membrane dynamics and a new form of astrocyte-neuron interaction.

Disclosures: M. Armbruster: None. **C.G. Dulla:** None.

Poster

611. Astrocytic Mechanisms of CNS Function

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Program #/Poster #: 611.18

Topic: B.09. Glial Mechanisms

Support: JSPS Grant (21F21733)

Title: Ultrastructural and functional study of the endoplasmic reticulum in perisynaptic astrocytic processes

Authors: *A. DENIZOT[1], M. VELOZ CASTILLO[3], P. PUCHENKOV[2], C. CALÌ[4], E. DE SCHUTTER[1];
[1]Computat. Neurosci. Unit, [2]Scientific Computing and Data Analysis section, Okinawa Inst. of Sci. and Technol., Onna-Son, Japan; [3]Biol. and Envrn. Sci. and Engin. Div., King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia; [4]Dept. of Neurosci., Univ. of Torino, Turin, Italy

Abstract: Neurotransmission at tripartite synapses results in Ca^{2+} signals in astrocytes, that are essential to brain function and impaired in brain disorders [1]. As most perisynaptic astrocytic processes (PAPs) are below the diffraction limit, their content in Ca^{2+} stores and the involvement of the latter in synaptic function remain unclear. Recent advances in electron microscopy (EM) allow to resolve the ultrastructure of astrocytes at an unprecedented spatial resolution. Here, we

reconstruct 3D meshes of tripartite synapses from a perfectly isotropic high-resolution (6 nm) 3D EM dataset and provide, in contrast to previous reports [2], unequivocal proof of the presence of the endoplasmic reticulum (ER), a major astrocytic Ca^{2+} store, in PAPs. Importantly, 75% of PAPs contained some ER, characterized by diverse geometrical properties. Stochastic reaction-diffusion computational models allow to study the causal relationship between cell geometry and cell signaling at the nanoscale. Simulations of our detailed biophysical model of astrocytic Ca^{2+} activity [3] in realistic PAP meshes extracted from EM revealed that PAP geometry changes are correlated with altered Ca^{2+} signals. To infer the causal relationship between individual geometrical features (*e.g.* ER surface area, ER distribution) and Ca^{2+} activity, we implemented an algorithm that alters ER distribution independently from ER and cell shape. Simulations in the resulting 3D PAP meshes revealed the complex interplay between the clustering of Ca^{2+} channels, ER surface-volume ratio, Ca^{2+} buffering and the size of ER-PM contact sites that shapes the spatio-temporal properties of Ca^{2+} signals in PAPs. Importantly, our results highlight that Ca^{2+} activity in realistic PAP shapes cannot be predicted by averaged models (*e.g.* cylinders), often used in computational studies as they require less computational resources and time. Overall, this study provides novel insights into the ultrastructural basis of astrocytic Ca^{2+} microdomain activity at tripartite synapses.
References : [1] Verkhratsky, A. & Nedergaard, M. Physiology of Astroglia. Physiol. Rev. 98, 239-389 (2018). [2] Patrushev, I., Gavrilov, N., Turlapov, V. & Semyanov, A. Subcellular location of astrocytic calcium stores favors extrasynaptic neuron-astrocyte communication. Cell Calcium 54, 343-349 (2013). [3] Denizot, A., Arizono, M., Nägerl, U. V., Soula, H. & Berry, H. Simulation of calcium signaling in fine astrocytic processes: Effect of spatial properties on spontaneous activity. PLOS Comput. Biol. 15, e1006795 (2019).

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Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.01

Topic: C.01. Brain Wellness and Aging

Support: Jump ARCHES 239

Title: Effect of Tai Chi on Beta-range Lower Limb Corticomuscular Coherence in Older adults

Authors: Y. HU, L. ZHAO, ***M. E. HERNANDEZ**;
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Abstract: Tai Chi practice (TCP) has been well documented in changing neuromuscular functions in older adults measured by electromyography (EMG) positively influencing cortical beta-range activities after exercise by electroencephalography (EEG). However, little is known

about the role of how cortical areas control muscle activity in older adults and adaption due to TCP. Coherence between EEG and EMG is thought to reflect corticospinal coupling between cortex and muscle motor units. Specifically, Beta-range (13-30 Hz) corticomuscular coherence (CMC) has been documented during static force output and isometric contractions. This study aimed to evaluate the beta-range CMC in standing virtual reality (VR) tests in older adults with and without TCP experience. We recruited five older adults with TCP (TCOA) and five no experience age-matched controls (OA). The testing paradigm included four trials, consisting of: 1) Seven levels VR height changes every 30 seconds; and 2) Ten randomized standing toe-down perturbations or no perturbation. 64-channel active electrode array EEG signals and eight ankle flexors and extensors EMG signals were recorded during the tests simultaneously. CMC was quantified by magnitude squared coherence (MSC) between EEG and EMG signals. CMC was significant if it was greater than the 95% confidence limit. Linear mixed effect models (LMM) were used to investigate the effects of cohort, conditions, muscle, and their interactions on CMC. To control for multiple comparisons, post-hoc Least-Squares Means tests were carried out using the Kenward-Roger method. Significant coherence between EEG-channel signals and lower limb EMG was observed in the beta-range for both groups in the frontal, center, parietal, and occipital areas. The coherence peaks occurred at 21.5 +- 5.32Hz and 21.9 +- 5.38Hz for OA and TCOA respectively. The LMM suggested statistically significant muscle, cohort-muscle interaction, and cohort-muscle-conditions interaction effects ($p<0.01$) on the MSC. Specifically, TC demonstrated significantly higher MSC than OA in left ankle dorsiflexors and right plantar flexors ($p<0.05$). While there are no differences in TCOA, OA has significantly higher MSC with both right ankle dorsiflexors and plantar flexors ($p<0.05$) when compared to the left side. In the TC group, both ankle plantar flexors and dorsiflexor MSC decreased as VR height increased and were perturbed while standing ($p<0.05$). Beta-range lower limb CMC were presented in challenging standing postural maintenance regardless of cortical region and TC experience, which might be explained and support the compensation theories of cortical control of posture in older adults.

Disclosures: Y. Hu: None. **L. Zhao:** None. **M.E. Hernandez:** None.

Poster

612. Brain Wellness and Aging

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.02

Topic: C.01. Brain Wellness and Aging

Support: Jump ARCHES Grant 239

Title: Effect of Tai Chi and Age on Beta-Band Power and Resting State Functional Connectivity

Authors: *M. HE[1], Y. HU[4,5], J. ZHAO[2], E. T. HSIAO-WECKSLER[3], M. E. HERNANDEZ[2]; [1]Neurosci., [2]Kinesiology and Community Hlth., [3]Mechanical & Industrial Engin., Univ. of

Illinois at Urbana-Champaign, Urbana, IL; [4]Kinesiology and Community Hlth., Univ. of Illinois at Urbana-Champagin, Urbana, IL; [5]Kinesiology, San José State Univ., San José, CA

Abstract: Introduction: Resting-state functional connectivity (rsFC) has been known to be altered as we age, and its disruption is often associated with cognitive decline in older adults. However, the underlying mechanisms behind these associations and the potential of physical activity interventions to ameliorate age-related rSFC disruptions remain unclear. Tai Chi, a Chinese traditional martial art, has been shown to have beneficial effects on both the cognitive and physical function of older adults, while its impacts on rsFC, and particularly beta band, may provide a distinct mechanism for observed behavioral improvements in older adults. **Aim**: The present study focuses on Tai Chi and its influence on rsFC in older adult practitioners (TCOA) in comparison with older non-practitioners (OA) and young adults control group (YA) using electroencephalography (EEG). Phase lag index (PLI) and percentage power (%power) of beta band are two main measures of rsFC. **Methods**: A total of 45 participants were recruited and grouped into three cohorts: YA (9 females, 6 males; 21.6 ± 2.1 years), OA (9 females, 6 males; 72.9 ± 4.8 years), and TCOA (10 females, 5 males; 77.1 ± 5.8 years). The present study involved sitting in a natural position while facing forward in two conditions: eyes closed (EC) and eyes open (EO). Each condition lasted for 60 seconds while resting state EEG data was collected. Cluster analysis was performed on preprocessed EEG data for four brain regions: frontal, middle, parietal, and occipital areas. PLI was calculated to evaluate rsFC for the beta band (13 - 30 Hz), and the %power was normalized to total power to reflect beta band power distribution. Linear mixed models were then applied to find whether there were any effects of cohort, task, and their interactions on PLI and %power. **Results:** The %power in the beta band significantly decreased in EC condition in both YA (6.51%) and TCOA (3.62%) groups compared to EO ($p < 0.01$). TCOA also had higher %power in the beta band compared to YA in both EO (7.52%, $p < 0.01$) and EC (10.41%, $p < 0.01$) tasks, and OA (9.64%, $p < 0.01$) in EO task. A similar but opposite task effect has been found for PLI that EC had higher PLI than EO (0.02, $p < 0.01$). Lastly, there was a trend that TCOA had higher PLI in the middle cortex regions when compared to YA (0.03, $p = 0.0532$) and OA (0.03, $p = 0.0544$). **Conclusion:** Aging may disrupt beta band regulation, while Tai Chi may have benefits in compensating for aging effects, especially in the motor-sensory area. The higher PLI but lower %power in EO could reflect increased beta synchronization may be masked by overall high synchronization in all bands during resting state with visual input, which merits future investigation.

Disclosures: M. He: None. **Y. Hu:** None. **J. Zhao:** None. **E.T. Hsiao-Wecksler:** None. **M.E. Hernandez:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.03

Topic: C.01. Brain Wellness and Aging

Title: Gait variability association with prefrontal cortical activation among older adults with and without impaired heart rate reserve

Authors: *A. BISHNOI[1,2], M. HERNANDEZ[1];
[1]Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Sch. of Physical Therapy, Kean Univ., Union, NJ

Abstract: Reduced gait variability and increased brain activation have been previously associated with dementia. Further, early cardiovascular changes in older adults, such as lower heart rate reserve (HRR) have been found to be linked with increased prefrontal cortical activation in older adults while dual task walking. Given that impaired HRR is predictive of future hypertension and cognitive decline, it is of importance to examine the potential link between gait variability and prefrontal cortical activity at an early stage in adults with impaired HRR. Impaired HRR affects cognitive function in older adults resulting in reduced motor and cognitive abilities. Dual task (DT) paradigms have shown the sensitivity to measure this cognitive decline while walking. Based on which, we have used the Instrumented trail walking task (TWT) to evaluate changes in cognitive function and gait variability in older adults with impaired HRR. This is the first study examining the effects of an instrumented TWT on gait variability and brain activation evaluated by functional near infrared spectroscopy (fNIRS) among older adults with and without lower HRR. We hypothesized that there would be a decrease in gait variability associated with increased brain activation among older adults going from easier to a more difficult task, especially in lower HRR adults. In this cross-sectional study, we examined 30 older adults (63.43±8.65yrs, 20 females). Participants completed baseline cognitive and motor testing before starting the paradigm on an instrumented treadmill. The DT paradigms consisted of four tasks: comfortable walking tasks (CWT) and trail walking tasks (TWT-A with only sequential numbers and distractors and TWT-B with both sequential letters and numbers and distractors). The order of the tasks was CWT, TWT-A, CWT, TWT-B. Participants completed the TWTs under their comfortable walking speed with easier task first (TWT-A) and difficult task later (TWT-B). We found significant differences in age and gait speed between LHRR and HHRR ($p<0.05$). The results of the correlation analysis showed that increased prefrontal cortical activation was associated with reduced gait variability ($p<0.05$) under TWT-A, especially driven by HHRR. We didn't find any other significant differences nor associations. The association between higher prefrontal cortical activation and reduced gait variability across all older adults, is consistent with recent findings. However, the differences in HRR groups need further exploration in a larger study.

Disclosures: **A. Bishnoi:** None. **M. Hernandez:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

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Program #/Poster #: 612.04

Topic: C.01. Brain Wellness and Aging

Title: Longitudinal analysis of mood for older adults in the Warfighter Analytics using Smartphones for Health (WASH) study

Authors: *S. BANERJEE[1,2], S. LI[3], R. HALABI[3], A. PRATAP[3], S. MADARASMI[1,2], P. PEDRELLI[1,2], J. E. CURTISS[1,2], F. A. JAIN[1,2];
[1]Depression Clin. and Res. Program, Dept. of Psychiatry, Massachusetts Gen. Hosp., Boston, MA; [2]Dept. of Psychiatry, Harvard Med. Sch., Boston, MA; [3]Krembil Ctr. for Neuroinformatics, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: It is estimated that 5% of adults experience depression or anxiety globally, and 5.7% are older adults. Rates of depression have increased due to economic downturns and the COVID-19 pandemic. Low mood is a cardinal symptom of depression that is mostly evaluated at monthly or longer intervals in office-based settings for clinical trials and mental health practices; however, these measurements can be confounded by retrospective bias and setting. Day-to-day variations in depressive symptoms including low mood may be assessed by collecting frequent self-report data via Ecological Momentary Assessments (EMA) in participant's natural environment and via sensors in mobile phones. Hence, smartphones can be leveraged to monitor individuals continuously, creating "personalized digital phenotypes" of psychological states such as low mood. However, older adults have been underrepresented in digital phenotyping studies. In this abstract, we primarily aim to identify significant predictors of low mood in older adults through smartphone sensor and survey data. Our sample, older adults aged 60 and above, is drawn from a large, 12-week observational study, Warfighter Analytics using Smartphones for Health (WASH), designed to obtain EMA of mood and other characteristics alongside continuous smartphone sensor data. Mood at baseline was measured with the Positive and Negative Affect Schedule scale (PANAS). Participants were binarized into low or high negative affect groups using 14 as a population mean cutoff score. We performed chi-square tests to examine the relationship between low mood at baseline and key attributes such as educational qualification, income, and self-reported physical activity. Out of 6715 participants completing baseline surveys, 386 older adults were identified (mean age of older adults = 66.23 years; σ = 4.67; approx. 59% female). Preliminary results to predict participants with high negative affect showed no significant relationship with education, income, or self-reported physical activity (p&gt.05 for all comparisons).To the best of our knowledge, this is one of the largest older adult samples in a study designed to capture digital markers of mental states with smartphones. The absence of strong predictors of mood at baseline further underscored a need for more advanced technological approaches. Further analyses will aim to create supervised machine learning approaches leveraging sensor data to predict low mood in older adults, towards the goal of improving measurement accuracy for research and clinical applications.

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Poster

612. Brain Wellness and Aging

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Title: Perturbed cerebral glucose uptake and glymphatic system in a mouse model of Huntington's Disease

Authors: *H. LIU[1], C. LIN[2], C. ZHANG[3], L. CHENG[4], J. HUA[5], C. LIU[3], C. A. ROSS[6], P. C. M. VAN ZIJL[5], J. XU[5], W. DUAN[7];
[1]Johns Hopkins Med. Institutions, Baltimore, MD; [2]Xiamen Univ., Xiamen, China; [3]Johns Hopkins school of medicine, Baltimore, MD; [4]NIH, Baltimore, MD; [5]Kennedy Krieger Inst., Baltimore, MD; [6]Div. of Neurobio., Johns Hopkins Med. Sch., Baltimore, MD; [7]Psychiatry, Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Division of Neurobiology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of medicine, Baltimore, Maryland, USA. [2]F.M. Kirby Research Center, Kennedy Krieger Research Institute, Baltimore, USA. [3] The Russell H. Morgan Department of Radiology and Radiological Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [4] Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. For Huntington's disease, the clinical diagnosis is currently based on the presence of movement disorders. However, subtle-to-prominent brain functional changes can precede by many years the motor-based onset of HD. Given the availability of predictive genetic testing, it is possible to identify HD subjects before motor symptoms are present. This provides a unique opportunity to identify potential early biomarkers to be used for evaluating the disease-modifying therapies. Glucose Chemical Exchange Saturation Transfer (glucoCEST) MRI is a newly developed technique that can detect unlabeled glucose at physiologically relevant concentrations using proton-only MRI scanners. Our recently improved MRI sequence, on-resonance variable delay multiple pulse (onVDMP), allows the detection of low concentration glucose through the chemical exchange of protons between hydroxyl groups and has the capacity to measure glucose clearance from the cerebrospinal fluid (CSF). In this study, we applied onVDMP MRI to measure glucose uptake in the brain and its clearance from the CSF after intravenous injection of D-glucose in the zQ175 HD mouse model. By comparing the DGE curves and maps, the glucose uptake in the striatum of HD mice (4.4 ± 1.35 %) was significantly reduced compared to the age and gender-matched control mice (7.16 ± 1.36 %, $p = 0.012$), suggesting the presence of an impaired glucose transporter at the blood-brain and blood-CSF barriers of zQ175 mice. Interestingly, a significantly slower glucose clearance from CSF was found in the zQ175 HD mice compared to the age and gender-matched controls by examining the CSF DGE signal at 30 min with respect to the signal at 10 min (DGE[30min -10min]=2.96± 1.7% for HD vs -1.7± 1.7% for WT, $p<0.05$). Further molecular study indicated decreased levels of water channel aquaporin-4 (AQP4, ~ 40% reduction in HD mice) in the striatum of zQ175 mice. These results imply a dysfunctional glymphatic system in the zQ175 HD mice. onVDMP MRI measures have the potential to be further developed as valuable biomarkers for HD.

Disclosures: **H. Liu:** None. **C. Lin:** None. **C. Zhang:** None. **L. Cheng:** None. **J. Hua:** None. **C. Liu:** None. **C.A. Ross:** None. **P.C.M. van Zijl:** None. **J. Xu:** None. **W. Duan:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.06

Topic: C.01. Brain Wellness and Aging

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NINDS RF1 NS122028
NIA P30 AG072946
NIGMS S10 OD023573

Title: Diffusion Tensor Free Water is Associated with Plasma Concentration of Neurofilament Light Chain and Cognitive Performance in Older Adults

Authors: *C. E. BAUER[1], C. PAPPAS[1], T. L. SUDDUTH LEE[3], D. M. WILCOCK[2], P. MAILLARD[4], A. CAPRIHAN[5], B. T. GOLD[1];
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Abstract: Free water (FW), which reflects unrestricted water diffusion in diffusion tensor imaging (DTI), has recently been proposed as a biomarker of cerebral injury and cognitive impairment. However, while FW is thought to be linked to neurodegeneration, this link has rarely been explicitly investigated. Here, we explored whether neurofilament light chain (NFL), widely accepted as a plasma marker of neurodegeneration, is associated with FW within tracts connecting the executive control network (ECN), which is a large-scale cognitive network sensitive to aging. We further explored whether FW within the ECN would likewise be associated with executive function performance. Fifty-three older adults (ages 65-85) without dementia were recruited and scanned at the University of Kentucky using a 3 Tesla MRI Siemens Magnetom Prisma with a 64-channel head coil. A 126-direction main DTI sequence using 4 b-values (0, 500, 1000, and 2000 s/mm^2), and a reverse phase-encoding direction DTI sequence were acquired. Free water (and FA / free water eliminated FA [FWE-FA]) was first calculated per voxel for each participant, registered to FMRIB space and then skeletonized using FSL's tract-based spatial statistics (TBSS). Skeletonized FW values were then averaged across the white matter tracts connecting the ECN using a previously defined fMRI template. Within one year of the scan date, all participants had blood plasma collected and completed the Uniform Data Set Version 3. Plasma NFL concentration (pg/ml) was assessed on the Quanterix HD-X instrument using the Simoa Nf-Light Advantage kit at a 1:25 dilution according to optimization performed by our biomarker core. Executive function was assessed for each participant using a

composite (digit span backwards and trails making part B test minus part A). Multivariate linear regression models controlling for age and gender were conducted in SPSS. Plasma NFL concentration was positively associated with FW in the ECN (p=0.038), but not with FA or FWE-FA in the ECN. Further, FW in the ECN was negatively associated with EF performance (p=0.018). Our results support the hypothesis that free water in large-scale cognitive networks are broadly driven by neurodegenerative processes, in contrast to FA, and that subsequent FW changes in these networks are linked with associated decreases in cognitive performance.

Disclosures: C.E. Bauer: None. **C. Pappas:** None. **T.L. Sudduth Lee:** None. **D.M. Wilcock:** None. **P. Maillard:** None. **A. Caprihan:** None. **B.T. Gold:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.07

Topic: C.01. Brain Wellness and Aging

Title: Brain-muscles connectivity during walk: use of the corticomuscular coherence to quantify the cognitive reserve

Authors: *V. LONGATELLI[1], L. CAFFI[1], S. BOCCIA[1], E. GUANZIROLI[2], F. MOLTENI[2], A. PEDROCCHI[1];
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Abstract: A detailed comprehension of central and peripheral processes underlying walking is essential to develop interventions to detect and compensate for gait decline occurring with age and conditions affecting the central nervous system. Recently, the combined use of electromyography (EMG) and electroencephalography (EEG) in the framework of coherence analysis has been established for neuromotor impairment assessment. Indeed, it provides insights into the functional connectivity between brain and muscles. In this study, we propose corticomuscular, intermuscular, and intramuscular coherences as measures of cognitive reserve (CR). CR can be defined as a process whereby a wider repertoire of cognitive strategies, as well as more flexible and efficient strategies, can moderate the manifestation of brain disease/damage. A method for gait-related CR quantification may be helpful to assess age and disease-related gait decline. We recorded 8 EEG signals and EMG signals of tibialis anterior and soleus muscles in 16 healthy young adults (≤ 30 years) and 13 healthy elderly (≥ 65 years) during three overground walking conditions: spontaneous walking, cognitive dual-task walking (counting backward by 7), and targeted walking (walking with targets drawn on the floor). We calculated corticomuscular, intermuscular, and intramuscular coherences using wavelet analysis. Data were averaged across gait cycles, identified with footswitches. The volume of significant coherence in the single support of the right leg (20-40% of gait cycle) and the beta band (13-30 Hz) was compared between groups and tasks. Median stride duration assessed gait performance. Corticomuscular

coherence and median stride duration increased for the elderly group in dual-task walking compared to spontaneous walking, while these increases were not detected in the young group. Comparing spontaneous and targeted walking, both groups significantly increased corticomuscular and intermuscular coherences, and median stride duration. During targeted walking, intermuscular coherence and median stride duration were significantly greater in the young groups than the elderly group. These results suggest age-related differences in CR that reflect different abilities to perform complex cognitive or motor tasks during gait. To complete dual-task walking, elderly subjects require more cortical involvement, which however fails to prevent kinematic adjustments (higher stride duration). Despite a significant increase in cortical involvement, low intermuscular coherence in old subjects prevents kinematic adjustments (higher stride duration) possibly required for precise execution of the task.

Disclosures: V. Longatelli: None. **L. Caffi:** None. **S. Boccia:** None. **E. Guanziroli:** None. **F. Molteni:** None. **A. Pedrocchi:** None.

Poster

612. Brain Wellness and Aging

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Program #/Poster #: 612.08

Topic: C.01. Brain Wellness and Aging

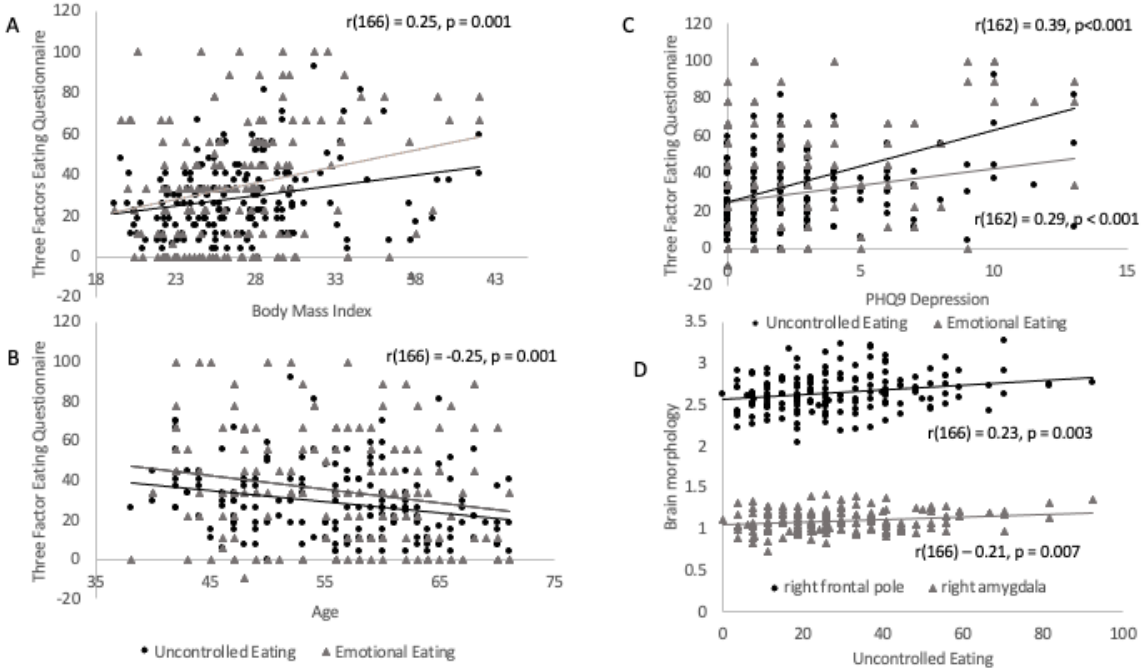
Support: Alzheimer's Society UK
BRACE Alzheimer's charity

Title: Psychological and neural correlates of eating behaviours

Authors: *C. METZLER-BADDELEY, J. MOLE;
Cardiff Univ., Cardiff Univ., Cardiff, United Kingdom

Abstract: Global obesity levels are on the rise with many associated health problems including Type 2 diabetes, cardio- and cerebrovascular disease, some types of cancer, and dementia[1]. To develop effective prevention and intervention strategies for obesity, it is important to gain a better understanding of the psychological and neural mechanisms that contribute to food-intake related behaviours. Here we explored the relationships between individual differences in eating behaviours measured by the 3-Factor Eating Questionnaire-R18[2] and differences in cortical thickness and subcortical volume measurements of the brain from FreeSurfer[3] in 166 community-dwelling adults from the Cardiff Ageing and Risk of Dementia Study[4,5] (38-71 years of age, 56% females). Linear regression models were employed to assess the brain predictors of cognitive restraint, uncontrolled eating, and emotional eating behaviours while controlling for the effects of age, sex and Body Mass Index (BMI) as well as psychological factors of depression measured with the Patient Health Questionnaire 9 (PHQ9)[6] and personality traits assessed with the Big Five Inventory[7]. Uncontrolled and emotional eating scores were positively correlated with BMI (Figure 1A) and depression scores (Figure 1C) and negatively with age

(Figure 1B). Emotional eating was more common in women than men ($t(164) = 3.2$, $p = 0.002$), but no sex difference was present for depression ($p = 0.6$). With regards to the neural mechanisms, differences in right pallidum volume contributed to cognitive restraint while differences in cortical thickness in the right frontal pole and right amygdala were significant predictors for uncontrolled eating (Figure 1D). These findings demonstrate that psychological and neural substrates of emotional dysregulation contribute to uncontrolled and emotional eating behaviours, which in turn may lead to obesity. These results accord with previous evidence suggesting that emotional dysregulation plays an important part in the development and maintenance of obesity[8].



Disclosures: C. Metzler-Baddeley: None. **J. Mole:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.09

Topic: C.01. Brain Wellness and Aging

Support: Collaborative Study on the Genetics of Alcoholism (COGA) U10 AA008401
Multi 'Omics Integration and Neurobiological Signatures of Alcohol Use Disorder (AUD) R01 AA027049
Integrating Epigenomics in Human Brain and Genomics of Nicotine Dependence

R01 DA042090
UK Biobank Resource under Application Number 48123
Washington University Institute of Clinical and Translational Sciences
TL1TR002344

Title: Alcohol, tobacco, and brain structure: common and substance-specific associations

Authors: *V. THORNTON[1,2], Y. CHANG[1], J. BIJSTERBOSCH[3], A. ANOKHIN[2], S. HARTZ[2], L. BIERUT[2];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Psychiatry, [3]Radiology, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Rationale: The UK Biobank is an unprecedented resource with self-reported alcohol use, smoking, genotypes, and 936 imaging derived phenotypes (IDPs). We leverage this opportunity to explore regions associated with alcohol use, pack years smoking, or both and to determine if there is an interaction between alcohol and smoking. Our analysis includes IDPs representing global and regional gray matter volume and cortical thickness derived from T1 MRI, white matter hyperintensity lesions derived from T2 MRI, white matter integrity from diffusion MRI, and 6 independent components from rfMRI.
Data: This study represents a subset of 35054 human participants between 40 to 80 years of age from the UK Biobank. The set includes both male and female participants.
Methods: Survey responses, genotyping, and MRI were all acquired in accordance with UK Biobank protocols. We performed linear regression in R to identify brain regions associated with alcohol use, pack years smoking, or both, as well as alcohol by smoking interaction while controlling for covariates (systolic and diastolic blood pressure, waist hip ratio, BMI, income, education years, pack years, brain volume, site, sex, age, imaging date, head size, and rfMRI motion). We used the chi squared test to determine if overlap between regions associated with alcohol and smoking is greater than expected by chance alone.
Results: Both alcohol and smoking are significantly (p less than or equal to 5.34*10^-5 after Bonferroni correction for multiple comparisons) associated with decreased brain volume, decreased grey matter volume, and decreased white matter volume. Smoking is associated with increased total volume of white matter hyperintensities, while alcohol is not. 102 out of 936 IDPs are significantly associated with alcohol use measured as drinks per week, while 242 are significantly associated with pack years. 42 (4.5%) of IDPs are significantly associated with both while 26 would be expected by chance alone. There is greater overlap in IDPs representing volumes from T1 MRI, and less in IDPs representing dMRI. We found no evidence of a significant alcohol by smoking interaction.
Conclusion: The overlap in IDPs associated with alcohol and smoking is greater than expected by chance, yet there are also many IDPs associated with one substance only. These results support both combined and independent mechanisms for the association between alcohol, smoking, and brain health. The lack of a significant interaction suggests these mechanisms are additive and not multiplicative.

Disclosures: V. Thornton: None. **Y. Chang:** None. **J. Bijsterbosch:** None. **A. Anokhin:** None. **S. Hartz:** None. **L. Bierut:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Patent 8,080,371,“Markers for Addiction”.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.10

Topic: C.01. Brain Wellness and Aging

Title: Drd2 c957t modifies relationships among tau pathology, gray matter volume, and memory

Authors: *C. J. CIAMPA[1], J. L. COWAN[1], S. M. LANDAU[2], R. LA JOIE[3], A. MURPHY[4], W. J. JAGUST[4], A. S. BERRY[1];
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Abstract: Preserved dopamine function has been linked with successful aging trajectories and may contribute to brain and cognitive reserve. Reductions in dopamine D2 receptors are related to memory impairments in Alzheimer's disease (AD), but there is a lack of research on whether optimal dopamine function contributes to slowing AD clinical progression. Higher D2 receptor availability has been linked with better episodic memory and greater cortical thickness. We leveraged data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) to probe relationships among genetically inferred differences in D2 receptor availability (DRD2 C957T; rs6277), cortical thickness, and memory function in the context of AD-related tau pathology. Our sample included 161 cognitively normal older humans (61-94 (M=74) years old, 86 female) who underwent MR scanning to measure cross-sectional cortical thickness, [18F]Flortaucipir ([18F]FTP) PET to assess entorhinal tau, genetic testing, and memory assessment (Rey Auditory Verbal Learning Test; RAVLT). Analyses included age, sex, and years of education as covariates. Consistent with previous reports (Miranda et al., 2021), we found the DRD2 T/T genotype, predicting higher D2 receptor affinity, was associated with greater cortical thickness and volume in temporal and parietal regions compared with DRD2 C/C homozygotes (p &lt .04). In line with previous work, higher entorhinal tau pathology was associated with reduced cortical thickness and volume in the temporal lobe. Critically, genotype had a moderating effect on this relationship, such that T/T individuals showed better-than-expected cortical thickness given entorhinal tau ($t(152) = 2.15$, $p = .03$, beta = 1.30, 95% CI = [.00006, .001]). Parallel analyses probing relationships with memory found higher entorhinal tau burden was associated with worse memory. DRD2 genotype interacted with entorhinal tau to predict RAVLT ($t(153) = 2.16$, $p = .03$, beta = 1.37, 95% CI = [2.01, 46.00]), where T/T subjects displayed better-than-expected memory given their tau burden. Together, these cross-sectional findings provide compelling preliminary evidence that higher D2 receptor affinity (DRD2 T/T) contributes to brain and cognitive reserve, allowing individuals to effectively cope with AD-related tau pathology. Future work will investigate the degree to which DRD2 T/T supports brain maintenance over time.

Disclosures: C.J. Ciampa: None. **J.L. Cowan:** None. **S.M. Landau:** None. **R. La Joie:** None. **A. Murphy:** None. **W.J. Jagust:** E. Ownership Interest (stock, stock options, royalty,

receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Jagust holds an equity interest in Optoceutics.. F. Consulting Fees (e.g., advisory boards); Dr. Jagust has served as a consultant to Biogen, Bioclinica, Roche/Genentech, and Grifols.. **A.S. Berry:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.11

Topic: C.01. Brain Wellness and Aging

Support: NIH-NIA Grant 1R43AG047722

Title: Cognitive reserve and control network connectivity explain variability in executive function processes.

Authors: *M. A. PIPOLY[1], H. LEE[2], E. HAZELTINE[3], M. VOSS[4];
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Abstract: Older adults vary widely in their executive function performance. Susceptibility to cognitive aging has been linked to Cognitive Reserve (CR), which has been proposed to develop from early life experiences such as education. CR is thought to reflect an adaptive mechanism protecting cognition against the adverse effects of brain aging. Recent work suggests CR is related to functional connectivity (FC) of brain networks and is most apparent with difficult tasks. Prior fMRI studies using executive function tasks show increased regional activity among brain regions that form the salience (SN), dorsal attention (DA) and frontoparietal (FP) networks termed, “control networks.” However, it is unclear whether CR affects control network FC during executive function performance beyond brain structural atrophy. I hypothesized greater control network FC would predict better executive function performance for individuals with higher educational attainment. Executive function was measured with a Flanker Task, where subjects (n=42, age=65-80) respond to target stimuli while ignoring adjacent distractors. I predicted control network FC for those with more education would show the strongest relationship to incongruent trials, where distractor stimuli indicated different responses than target stimuli, as these trials are thought to require greater attentional control. Additionally, I predicted control network FC for those with more education would predict smaller trial to trial changes in congruency effects, where the congruency of the immediately preceding trial affects the magnitude of the congruency effect on the current trial. To assess whether the hypothesized CR interaction was specific to control networks, I examined these same relationships with Default Mode Network FC. I first tested a three-way interaction among education, network FC, and task condition (congruent/incongruent/neutral) on reaction time while adjusting for age, sex, and cortical thickness. Next, I tested the two-way interaction among education and network FC

on the congruency sequence effect while adjusting for age, sex, and cortical thickness. Results showed greater SN-FC predicted better incongruent trial performance (*t(4833)=-3.41,p<.001)* when subjects had more years of education. Additionally, greater FPN-FC predicted a small influence of prior trial congruence (t*(34.26)*=-2.57,p=.01) when subjects were more educated. We conclude CR indicators like education may modulate SN and FPN-FC differently to buffer high performance demands among cognitively normal older adults.

Disclosures: M.A. Pipoly: None. **H. Lee:** None. **E. Hazeltine:** None. **M. Voss:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.12

Topic: C.01. Brain Wellness and Aging

Support: CIHR operating grant 406873

Title: Elevated biomarkers of neural injury observed in older adults following 14 days of six-degree head-down tilt bed rest

Authors: *A. P. BLABER[1], I. A. SCARISBRICK[2];
[1]Biomed. Physiol. and Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada; [2]Mayo Clin., Rochester, MN

Abstract: Introduction: Spaceflight and its ground-based analog, 6-degree head-down tilt bed rest (HDBR), results in whole-body deconditioning similar to aging. Along with cardiac and skeletal muscle atrophy, bone loss, and insulin resistance, recent evidence points to possible reflex neural impairment of blood pressure and skeletal muscle control. We hypothesized that inactivity and the headward fluid shift associated with HDBR would produce conditions promoting neural injury. Methods: The 14-day HDBR study was conducted at the McGill University Health Centre Research Institute. Ethical approval was obtained from the research ethics boards of MUHC and Simon Fraser University. Twenty-two healthy men (n=11), and women between 55 - 65 years of age (59.2±2.9 y) participated. A standard venipuncture was performed two days before HDBR (BDC-2), on the ninth day of HDBR, and on the first day of recovery (R0) from the antecubital vein in the arm into appropriate tubes. Thereafter blood was centrifuged, and blood sera were frozen in liquid nitrogen and stored at -80°C until analyzed. Serum samples were shipped via overnight courier on dry ice to the Wellington Laboratory at the Djavad Mowafaghian Centre for Brain Health at the University of British Columbia for independent analysis. Single Molecular Array (Simoa® , Quanterix Corp., USA) analysis was performed on the sera for Tau (Tau protein), NfL (neurofilament light chain), GFAP (glial fibrillary acidic protein), TNF-α (Tumor necrosis factor alpha), and IL-6 (interleukin 6). Repeated measures ANOVA was used to compare effects of HDBR and biological sex. Results: No differences were found between participant biological sex and response to HDBR for any of

the biomarkers. There was no change in Tau protein over the course of HDBR ($p=0.408$). NfL increased 36% from the beginning to the end of bed rest ($p<0.0001$) with most of the change occurring by HDBR 9. GFAP increased 21% from the beginning to the end of bed rest ($p<0.0001$). TNF-α also rose by 22% with bed rest ($p=0.006$) and IL-6 increased significantly at HDBR 9 where it plateaued at 40% ($p=0.0006$) above baseline. Conclusions: These data suggest that even a short period (9-14 days) of HDBR was sufficient to produce significant elevations of major protein markers of neural injury. This raises significant concerns regarding post-bed rest recovery and possible long-lasting effects, particularly in the elderly. These results also highlight the need for neurological monitoring of astronauts as space missions become longer in duration.

Disclosures: A.P. Blaber: None. **I.A. Scarisbrick:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.13

Topic: C.01. Brain Wellness and Aging

Support: Psychiatry Research Funds for Southern Region of Denmark

Title: Age-related changes in cerebral blood flow, glucose metabolism, and amyloid plaques density in AD signature regions and default mode network of healthy subjects: A PET/MRI fusion study

Authors: *M. SEYEDI VAFAEE, L. VIND KNUDSEN, T. MICHEL;
Psychiatry Res. Unit, Univ. of Southern Denmark, Odense, Denmark

Abstract: Purpose: We intended to test a chain of hypotheses that link a multitude of neurobiological mechanisms into a single concept of brain aging and by that to investigate the process of healthy aging by measuring the neurobiological parameters affected during the process of aging.
Hypothesis: Cerebral glucose metabolism (CMRglc) declines in healthy aging. The decline not only is associated with decreased aerobic glycolysis and cerebral blood flow (CBF) but also with age-dependent deposition of amyloid plaques. Furthermore, the amyloid plaque deposition is negatively correlated with white matter integrity.
Introduction: Alzheimer´s disease (AD) most commonly affects older adults and worsens with the age. In the context of studying the physiological parameters affected in AD, the current study aimed to investigate the process of healthy aging, i.e., the natural changes in physiological parameters that start at the onset of adulthood.
Materials and methods: 90 (50 reported) healthy subjects aged from 20-80 years (mean: 43.29 SD 16.40) in 6 groups of aging. underwent two sessions of PET and simultaneous MRI scans with the state-of-art SIGNA PET/MRI tomography. We first measured the amyloid plaque density with ^{11}C-PiB tracer which was followed by a ^{18}F-FDG scan for the measurements of

CMRglc. CBF was measured by means of ASL (arterial spin labelling) sequences which was acquired simultaneously by MRI. White matter track density was also measured by Diffusion Tensor Imaging (DTI).
Results: CBF and CMRglc declined significantly in the hippocampus, MTL, PFC (known as parts of the so-called AD signature regions) as well as in the default mode network (PCC, mPFC, precuneus), as a function of aging, i.e., from younger age group to the older groups. Conversely, amyloid plagues density increased in the same regions as the subjects ages increased. Fractional anisotropy (FA), a measure of the directionality of Brownian motion, also decreased with age at the same regions, insinuating decreased white matter integrity. The results also revealed a positive correlation between FA and CMRglc.
Conclusions: During the process of healthy aging, the main neuronal energy source i.e., glucose and most likely oxygen which are supplied by blood flow decline in regions known to be associated with AD while the density of AD related protein amyloid increases with a certain stoichiometry. Moreover, the integrity of white matter also was compromised in these regions with positive correlation with glucose metabolism. We hope our follow-up study with AD patients will demonstrate that the disturbance in that stoichiometry is indeed one of the main reasons for the pathogenesis of AD.

Disclosures: **M. Seyedi Vafaee:** None. **L. Vind Knudsen:** None. **T. Michel:** None.

Poster

612. Brain Wellness and Aging

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.14

Topic: C.01. Brain Wellness and Aging

Support: NIH grant DK082370
NIH grant DK082342
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NIH grant DK082344
NIH grant DK082325
NIH grant DK082345
NIH grant DK082316

Title: Chronic pelvic pain is not associated with accelerated brain aging: a 3-year longitudinal study from the MAPP Research Network

Authors: *K. A. LEECH, S. X. FAN, E. GASMIN, J. J. KUTCH;
USC, USC, Los Angeles, CA

Abstract: The presence of chronic pain is well-known to cause changes in brain structure and function that may be consistent with those observed in healthy aging. This suggests that chronic pain may accelerate brain aging. Methods have recently been developed to estimate the

biological age of the brain (i.e., brain age) through the application of machine learning to T1-weighted structural magnetic resonance imaging data. To date, a limited number of cross-sectional studies have applied these methods to investigate the relationship between chronic pain conditions and brain age. The results remain inconclusive. In this study, we applied a pre-trained brain age prediction algorithm (BrainageR) to structural neuroimaging data of 484 people with Urologic Chronic Pelvic Pain Syndrome (UCPPS) and 65 individuals without chronic pain at multiple timepoints. This was a secondary analysis of a dataset generated through collaborative work in the Multidisciplinary Approach to the study of Pelvic Pain Neuroimaging Working Group collected across 6 study sites. We aimed to 1) examine the test-retest reliability of brain age predictions, 2) examine the sensitivity of the measure over 3 years, and 3) determine the ability to separate individuals with UCPPS from healthy controls based on brain age. Estimates of brain age taken approximately 6 months apart showed excellent test-retest reliability (ICC (95%IC) = 0.93(0.92-0.94); n=420). A mixed-effect linear model demonstrated that over three years, brain age prediction values increased over time ($p<0.001$ for the time fixed effect). Interestingly, we were not able to distinguish people with UCPPS from healthy controls based on brain age values at baseline ($p=0.95$ for the group fixed effect), indicating that there is no relationship between the presence of UCPPS and brain age. This suggests the presence of chronic pain affects specific brain circuits rather than stimulating more systemic processes that accelerate brain aging.

Disclosures: **K.A. Leech:** None. **S.X. Fan:** None. **E. Gasmin:** None. **J.J. Kutch:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.15

Title: WITHDRAWN

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

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Program #/Poster #: 612.16

Topic: C.01. Brain Wellness and Aging

Support: NIH NINDS 5R01NS109529-04

Title: Assessing glutamate and glutamine in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): a magnetic resonance spectroscopy study

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Abstract: Background: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) involves chronic ongoing fatigue with unknown pathophysiology. Previous studies have linked abnormal glutamate (Glu) and glutamine (Gln) metabolite concentrations to fatigue. The relationship between Glu, Gln, and fatigue in ME/CFS has yet to be determined. Magnetic resonance spectroscopy imaging (MRSI) is commonly used to assess metabolite concentrations in the brain. Only a few studies have investigated Glu and Gln metabolite concentrations in ME/CFS patients, and these studies used single-voxel MRS. In this study, by collecting multi-voxel MRS information across the brain, we can expand our knowledge of these metabolite concentrations in ME/CFS. This study aimed to determine if concentrations of Glu and Gln in the brain are associated with ME/CFS symptomology.Methods: Twenty women with ME/CFS and twenty age-matched healthy women completed whole-brain MRS imaging and fatigue questionnaires. Glu and Gln were evaluated in 47 regions of interest (ROIs) as ratios over creatine (Cr). Independent samples t-tests compared ME/CFS patients and healthy controls. Bivariate correlations were also conducted to evaluate the ME/CFS group Glu and Gln with fatigue severity scale (FSS) score. Results: Our preliminary findings indicated significant metabolite variations between groups in 6 of 47 ROIs at $p < .05$. The ME/CFS group showed higher Gln/Cr than the healthy controls in the right supplementary motor area. ME/CFS participants had higher Glu/Cr in the right rolandic operculum, right fusiform gyrus, left pallidum, left temporal cortex, and right occipital cortex than healthy controls. We also found that a higher FSS score in ME/CFS patients was significantly correlated with Glu/CR in the left thalamus, left hippocampus, and right caudate. Additionally, we found FSS positively correlated with Gln/Cr in the right frontal cortex, left hippocampus, right caudate, and the left thalamus.Conclusions: These preliminary findings suggest that ME/CFS involves the dysregulation of Glu and Gln. Higher Glu is believed to be involved in pain, mood disturbances, and concentration issues, which are common symptoms associated with ME/CFS. These findings warrant further study in a larger sample.

Disclosures: I.A. Jordan: None. **S.N. Fox:** None. **M.L. McDaniel:** None. **S. Sheriff:** None. **J.W. Younger:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.17

Topic: C.01. Brain Wellness and Aging

Support: Norwegian Advisory Unit for fMRI
Central Norway Regional Health Authority

Title: Hippocampal metabolite profile in older adults after three years of supervised exercise versus following the national physical activity guidelines: proton spectroscopic imaging in the Generation 100 Study

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Abstract: Introduction: Physical activity and exercise are thought to preserve brain structure and function in older adults. Effects of physical activity/exercise on brain metabolites have not been reported. In this study we assessed the N-acetyl aspartate (NAA)/Creatine (Cr) and Choline (Cho)/Cr ratios in the right hippocampal head and body in older adults in the randomized control trial Generation 100 Study. We predicted that the supervised exercise group (SEG) had a higher NAA/Cr level than the control group (CG) after 3-years. Based on the cardiovascular fitness hypothesis, we also predicted that a greater increase in oxygen uptake (VO_2) from baseline to 3-years was associated with higher NAA/Cr levels. Methods: Participants were from the general population, born between 1936-42. Exclusion criteria were diseases precluding physical activity and MRI incompatibility. The SEG performed twice weekly medium intensity continuous or high intensity interval training. The CG followed the Norwegian physical activity guidelines of >30 minutes of moderate physical activity ≥5 days a week. Sociodemographic, VO_2 and clinical variables were collected at baseline and 3-years. MRI/MRS was acquired at 3T. A high-resolution 3D T1-weighted scan and a 2D chemical shift MRS sLASER sequence were used. Right hippocampal head and body volumes were obtained with FreeSurfer and NAA/Cr and Cho/Cr ratios in voxels in the hippocampal head and body obtained with LCModel. General linear models were used to assess group differences and associations. Sex, age, education, and hippocampal head/body volume were included as covariates. Results: Usable MRS data was obtained in 32 SEG and 31 CG participants. Both groups adhered well to their training and SEG trained at a higher intensity. SEG had significantly lower NAA/Cr ratio in the hippocampal body than the CG ($p=.04$, $B=.19$, $R^2=.19$). Across all participants, no associations between increase in VO_2 or VO_2 at 3-years and hippocampal metabolites were found, but a higher training intensity at 3-years was associated with lower Cho/Cr level in hippocampal body ($p<0.001$, $B=.58$, $R^2=.30$). Discussion: Contrary to our prediction, SEG had lower NAA/Cr levels in the hippocampal body after 3-years than CG and increasing or a high VO_2 was not associated with higher NAA/Cr levels. Higher exercise intensity was associated with lower Cho/Cr levels across all participants. Our results suggest that following the national physical activity recommendations preserved hippocampal neuronal metabolites the best and that training at a high intensity might be associated with loss of cellular membranes pointing to training at a lower intensity as preferable in older adults.

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Poster

612. Brain Wellness and Aging

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Program #/Poster #: 612.18

Topic: C.01. Brain Wellness and Aging

Support: DOD Research and Education Program
US Army Research Office and the xTech HBCU Program
UNC-Pembroke Faculty Research Grants

Title: Investigating cytoskeletal and adhesion dynamics associated with dementia risk factors arising from toxin exposures and seizure-related neuronal activity

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Abstract: Many risk factors have been identified that influence a person's vulnerability to dementia, especially age-related dementias which are escalating to a crisis of the 21st century. We have continued our comparative analyses to address whether commonalties exist with regards to altered cytoskeletal and adhesion dynamics among the different brain vulnerabilities linked to dementia risk. Exposure to organophosphate (OP) acetylcholinesterase inhibitors, used in pesticides and as warfare agents, is known to cause increased risk in developing long-term neurological disorders. Using an OP toxin exposure model in brain explants (Farizatto et al. 2019 Scientific Rpts 9:6532), we found distinct synaptopathy produced in addition to astrocytic activation and altered structural dynamics, as compared to the control cultures exhibiting well-maintained hippocampal tissue. The OP proconvulsant treatment using paraoxon (Pxn) led to oxidative stress and lasting spectrin breakdown mediated by calpain, similar to the cytoskeletal compromise found to correspond with seizure-associated excitotoxicity (Karanian et al. 2007-JPET 322:1059; Naidoo et al. 2012 Neurotherapeutics 9:801). The Pxn-induced spectrin fragmentation correlated with synaptic marker decline, and the Pxn insult also disrupted plasticity-related actin dynamics related to p-Cofilin activation. It is of interest that military blasts, from explant studies that showed dementia-related synaptopathogenesis initiated by the blast exposures, did not cause evident spectrin breakdown but caused neuropilar reduction in neural cell adhesion molecule NCAM180 as well as NCAM180 proteolytic breakdown (Smith et al. 2016 Exp Neurol 286:107; Almeida et al. 2021 Brain Pathology 31:e12936). Pxn exposures, on the other hand, did not alter NCAM180. The adhesion dynamics triggered by the Pxn insult rather consisted of a distinct increase in β1 integrin, whereas no quantitative changes were detected for NCAM180 or neurexin. Note that Pxn-mediated synaptopathy was not evident when the insult was applied after brain explants were treated with the β1 integrin inhibitor BIO 5192, thus supporting the idea that integrin responses play a role in governing synaptic vulnerability and toxicity. These comparative assessments indicate that while OP toxin exposures and other military-related vulnerabilities have been linked to an increase in dementia risk later in life,

disparate impacts on cytoskeletal and adhesion chemistries in the brain are evident among the different risk factors.

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Poster

612. Brain Wellness and Aging

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Topic: C.01. Brain Wellness and Aging

Support: NIH Grant P01AG055367
NIH Grant R01AG076838

Title: Never smokers' protection from white matter damage is minimized with high exposure to air pollution

Authors: *C. FENNEMA-NOTESTINE[1], J. A. ELMAN[2], L. K. MCEVOY[3], R. NOTESTINE[2], X. TU[4], W. S. KREMEN[2], C. E. FRANZ[2];
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Abstract: Although long-term exposure to air pollution has been associated with vascular disease in older adults, it remains unclear whether white matter damage (e.g., white matter hyperintensities, WMH) is clearly linked to pollutant exposure particularly within middle age. White matter damage is characterized by factors such as demyelination, inflammation and gliosis, and WMH are associated with aging, hypertension, and worse cognition. In our Vietnam Era Twin Study of Aging (VETSA), we defined WMH through a multi-channel tissue segmentation approach, demonstrating WMH predominantly in periventricular and deep parietal and frontal regions in middle age. Using multivariable linear regression, we examined the association between WMH volumes and average residential air pollution exposure to PM2.5 (particulate matter with aerodynamic diameter <2.5μm) over 36 months just prior to each acquired MRI scan in 194 men (mean age 62, range 56-67). To address additional pollutant exposure, we included smoking status, categorized as never (n=78), former (n=84), or current (n=32), and the interaction of smoking status and PM2.5 exposure. We controlled for age, race/ethnicity, other environmental exposures (e.g., pesticides, industrial metals), and family as a random factor to account for correlated observations within twin pairs. Global and regional WMH volumes were adjusted for total white matter volume. Regional WMH volumes were measured with a novel, data-driven parcellation method using our VETSA WMH frequency atlas and a watershed algorithm to define five subregions: 1) posterior, 2) superior frontal and parietal, 3) anterior/inferior frontal and deep, 4) occipital, and 5) anterior periventricular. Although no significant main effects of PM2.5 or smoking status were found, the interaction of smoking status and PM2.5 exposure was significant for global WMH (p=.007) and within posterior

(p=.02), superior frontal and parietal (p=.002), and anterior/inferior frontal and deep (p=.01) regions. The interaction was not significant for occipital or anterior periventricular regions. Never smokers had smaller WMH volumes in these regions relative to current smokers but only with low PM2.5 exposure levels. When PM2.5 exposure was high, WMH volumes were similarly higher for never, former, and current smokers. By not smoking, it is possible to substantially avoid cigarette smoke, while avoiding air pollution is difficult. Our results showed that protection from white matter disease in individuals who never smoked was minimized in high PM2.5 exposure environments.

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Poster

612. Brain Wellness and Aging

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Topic: C.01. Brain Wellness and Aging

Support: NIH/NIA Grant 1P30AG066508-01
NIH/NIA Grant 5K23AG059919-04
AAIC Grant 2019-AACSF-644153

Title: Identification of significant connectivities that robustly predict memory performance in healthy aging adults using connectome-based predictive modeling

Authors: *S. JU[1], C. HORIEN[2], R. T. CONSTABLE[3], C. A. FREDERICKS[1];
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Abstract: Alzheimer's disease (AD) has been associated with characteristic changes in brain connectivity and, in particular, targets an intrinsic connectivity network (ICN) known as the default mode network (DMN). A growing literature in aging adults also suggests that the relationships between ICNs are important, as desegregation of networks due to aging correlates with poorer memory performance and increased risk of dementia. To pinpoint aspects of connectivity both within and beyond the DMN, we identified specific edges that robustly predict memory performance (measured by the Rey Auditory Verbal Learning Test (RAVLT) sum-of-trials scores) by leveraging data-driven machine learning to model the brain connectome in the Lifespan Human Connectome Project Aging (HCP-A) dataset (Harms et al. 2018). We created whole-brain connectivity matrices based on the Shen 268 node parcellation (Shen et al. 2013) from functional MRI scans of 725 healthy subjects (319 men, 406 women) aged 36 to 100. Connectome-based predictive modeling (CPM) with p-value threshold of 0.01 and 5-fold cross-validation was used to train predictive models, which were then applied to test sets, resulting in predicted RAVLT measures and Pearson correlations between predicted and observed scores

across all subjects for each of 7 different scan types (4 resting-state, 3 task-based). Positive and negative-edge matrices describing all node connectivities were analyzed using the BioImage Suite Web Connectivity Viewer tool. Internetwork connectivity strength was calculated by dividing the number of significant edges between each network pair by the number of total edges between the two networks. As anticipated, the highest number of significant edges occurred most within the DMN. Across all scan types, DMN nodes (particularly anterior prefrontal cortex (PFC), posterior cingulate cortex (PCC), and orbitofrontal cortex) and visual network (VN) nodes (in the primary (VI) and secondary (VII) visual cortices, visual associative (VA) cortex, and cerebellum) were among the nodes with the highest degrees. Memory performance was associated with positively-correlated edges within the DMN at rest and with negatively-correlated edges between VN-DMN during tasks requiring visual processing. During these tasks, memory performance was also associated with negatively-correlated edges between DMN-limbic network (LN), which suggests segregation between DMN-LN during task performance may associate with higher memory scores. We successfully implemented CPM to derive robust brain-based predictors of memory performance and identify the connectivities that most contributed to our models' predictions.

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Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

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Program #/Poster #: 612.21

Topic: C.01. Brain Wellness and Aging

Title: Characterizing demyelination in models of aging and CNS dysfunction

Authors: *J. H. SIN, C. WANDER, I. GALLAGER, E. CZIRR, C. YANG;
Alkahest, San Carlos, CA

Abstract: Characterizing demyelination in models of aging and CNS dysfunction Johnny Sin, Connor Wander, Ian Gallager, Eva Czirr, Cindy Yang
White matter degeneration is a critical component of aging, as the ability to repair and replace healthy cells that promote the normal myelin renewal process decreases over time. Maintaining myelin sheath integrity is important to ensure proper axonal function and efficient signal transduction in neurons, and loss of white matter contributes to cognitive impairment, specifically memory consolidation, in many neurodegenerative conditions. Here we outline multiple *in vivo* mouse models with impairments in myelination and evaluate their potential utility for testing preclinical therapeutic efficacy. We compared the coverage of myelin basic protein (MBP) across various models, such as experimental autoimmune encephalomyelitis, high fat diet, hyperhomocysteinemia, and aging, and correlated myelin loss with readouts of neuroinflammation and blood brain barrier leakage. We also quantified differentiating oligodendrocyte precursor cells by measuring neural-glial antigen 2 (NG2) expression to capture

the remyelinating capacity. Further, we characterized the extent of white matter damage in the hippocampus, cortex, and hypothalamus, thereby establishing a pattern of demyelination in these models of cognitive dysfunction. We propose that these mouse models could be used to evaluate potential therapeutics aimed at restoring myelin levels in disease.

Disclosures: **J.H. Sin:** A. Employment/Salary (full or part-time):; Alkahest. **C. Wander:** A. Employment/Salary (full or part-time):; Alkahest. **I. Gallager:** A. Employment/Salary (full or part-time):; Alkahest. **E. Czirr:** A. Employment/Salary (full or part-time):; Alkahest. **C. Yang:** A. Employment/Salary (full or part-time):; Alkahest.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.22

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant P01 AG055367
NIH Grant K01 AG063805

Title: Association of PM2.5 exposure and MRI-assessed locus coeruleus integrity

Authors: *J. A. ELMAN[1], O. K. PUCKETT[1], C. FENNEMA-NOTESTINE[1], D. J. HAGLER, Jr.[2], W. S. KREMEN[1], C. E. FRANZ[1];
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Abstract: Increased exposure to air pollution, and exposure to PM2.5 (particulate matter with aerodynamic diameter &lt2.5μm) in particular, has been associated with worse brain health and increased risk for dementia. The locus coeruleus (LC) is a brainstem structure with diffuse projections throughout the brain. It is an initial site of tau deposition and its dysfunction may contribute to disease progression. Due to its high exposure to the cerebrovascular system and proximity to the 4th ventricle, the LC may be particularly vulnerable to environmental toxins. Here, we examine the relationship between PM2.5 and in vivo estimates of LC structural integrity. A total of 398 participants (mean age = 67.3) from the Vietnam Era Twin Study of Aging (VETSA) were included. Rostral/middle LC structural integrity was indexed by contrast-to-noise ratio (LC_{CNR}) from an LC-sensitive MRI scan. Air pollution data from place of residence were collected for the average 3-year level of PM2.5 from the most recent period of measurement (ending an average of 5.6 years prior to MRI scan). Our focus was on rostral/middle LC because it more strongly associated with aging and Alzheimer's disease than the caudal LC. We performed mixed effects models with random intercept to account for correlated outcomes within twin pairs with rostral/middle LC_{CNR} as dependent variable and PM2.5 as independent variable. Models were adjusted for age, race/ethnicity, number of other environmental exposures and scanner. In separate models, caudal LC_{CNR} and hippocampal volume were used as dependent variables to assess regional specificity of associations. There

was a significant association between the 3-year average of recent PM2.5 and rostral/middle LC_{CNR} (β=-0.12, p=0.02) whereby higher exposure to PM2.5 was associated with lower LC_{CNR}. There were no associations between PM2.5 and caudal LC_{CNR} or hippocampal volume. Exposure to PM2.5 in early old age is associated with lower LC integrity. Taken together with previous findings suggesting reduced LC integrity may exacerbate AD-related processes, this may represent one pathway through which air pollution negatively impacts brain health and increases risk for dementia.

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Poster

612. Brain Wellness and Aging

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Mt. Jade Young Scholarship Award from Ministry of Education, Taiwan
National Yang Ming Chiao Tung University and the Ministry of Education (Aim for the Top University Plan), Taipei, Taiwan

Title: Exploring the variations in the functional network of the aging brain by decomposing blood-oxygen-level-dependent signals

Authors: *W.-X. TSAI, A.-C. YANG;
Inst. of Brain Science, Col. of Medicine, Natl. Yang Ming Chiao Tung Univ., Taipei City, Taiwan

Abstract: Functional brain networks are typically measured by Pearson's correlation of blood-oxygen-level-dependent (BOLD), which has been used in many studies as indirect evidence of dynamic brain activity. Previous aging-related studies have demonstrated the association between functional brain networks and aging. However, the correlation between BOLD signals, which was a nonlinear signal, may be affected by the instantaneous amplitude and instantaneous phase of the signals. In addition, few studies have investigated the correlation between different BOLD sub-signals and their potential meaning. Because of this, to understand more about the information hidden in the BOLD signals, this study aimed to explore a novel approach to brain

network building. Also, to investigate the changes in information transmission efficiency of brain networks between different ages. A total of 399 healthy participants between the ages of 20 and 81 were enrolled. After acquiring functional brain images, we adopted ensemble empirical mode decomposition (EEMD) to decompose BOLD signals into several intrinsic mode functions (IMFs). The Pearson's correlation network, amplitude correlation network, and phase coherence network were then created based on each type of signal. Consequently, 12 functional networks were built based on different signals and correlation types. We quantified the information transmission efficiency of different networks using the concept of small-world networks. Finally, the global efficiency and local efficiency of functional brain networks in different networks were used to build regression models for predicting age.The results showed the correlation between age and global efficiency in 7 out of 12 functional brain networks, including Pearson's correlation networks based on BOLD signals and IMF2, amplitude correlation networks based on BOLD signals and IMF1, and phase coherence networks based on BOLD signals, IMF2 and IMF3. In terms of local efficiency, 8 out of 12 functional brain networks were found to exhibit age-related brain regions, including Pearson's correlation networks based on BOLD signals and three decomposed IMFs, amplitude correlation network based on BOLD signals, and phase coherence networks based on BOLD signals, IMF1 and IMF2. Most of the results showed a difference from the functional network created by the traditional approach. Our findings suggest that functional brain networks created based on decomposed BOLD signals and different correlation types can shed light on the aging-related functional brain networks in different spatial scales.

Disclosures: W. Tsai: None. **A. Yang:** None.

Poster

612. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 612.24

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NS097805
NS37853

Title: Intranasal administration of tissue plasminogen activator restores neurovascular and cognitive dysfunction in a mouse model of amyloid pathology

Authors: K. UEKAWA[1], A. ANFRAY[2], J. SEO[2], N. CASEY[2], P. ZHOU[2], C. IADECOLA[2], ***L. PARK**[2];
[1]Dept. of Neurosurg., Kumamoto Univ. Hosp., Kumamoto, Japan; [2]BMRI, Weill Cornell Med., New York, NY

Abstract: The amyloid-β peptide (Aβ) has profound neurovascular effects that may contribute to the cognitive dysfunction underlying Alzheimer's dementia (AD). Aβ attenuates the increase in

cerebral blood flow (CBF) evoked by neural activity (functional hyperemia). Functional hyperemia requires tissue plasminogen activator (tPA) which enables the NMDA receptors (NMDAR)-dependent component of the response (PNAS., 105:1076, 2008). In a model of amyloid pathology (Tg2576 mice) we have previously shown that tPA deficiency contributes to neurovascular and cognitive dysfunction (J Neurosci., 40:8160, 2020). Therefore, we investigated whether restoring brain tPA levels by intranasal tPA administration ameliorates neurovascular and cognitive function in aged Tg2576 mice. Male Tg2576 mice and non-transgenic (WT) littermates (8-10 mice/group; age 12 months) were instilled intranasally with recombinant tPA (rtPA; 20 µg/day; 5 days/week) or vehicle (veh; 10µl dH_2O/nostril) and were examined 3 months later. In veh-treated tg2576 mice, resting neocortical CBF, measured by ASL-MRI, was attenuated (-22%) compared to veh-treated WT littermates (121±7 vs. 95±4 ml/100g/min; $p<0.05$). The CBF reduction was completely reversed by treatment with rtPA (resting CBF: 118±5 ml/100g/min; $p>0.05$ from WT). To test the effect of rtPA on neurovascular coupling, CBF was monitored by laser-Doppler flowmetry via a cranial window over the somatosensory cortex of urethane-chloralose anesthetized mice. In veh-treated Tg2576 mice, the increase in somatosensory cortex CBF produced by neural activity evoked by whisker stimulation was attenuated (-40%) compared to veh-treated WT mice (19±2 vs. 11±1%; $p<0.05$). Likewise, the CBF increase elicited by neocortical application of NMDA (40 µM) was reduced in Tg2576 mice (-23%) ($p<0.05$ from WT). Intranasal rtPA rescued the attenuation of the CBF response to both whisker stimulation and NMDA application ($p<0.05$ from veh-treated Tg2576 mice). Nest building behavior was reduced in veh-treated Tg2576 mice (nesting score: -40%; $p<0.05$), indicating cognitive dysfunction. Intranasal rtPA normalized nesting behavior in Tg2576 mice ($p>0.05$ from veh-treated WT). The data suggest that restoring tPA counteracts the deficits in the NMDAR-dependent component of functional hyperemia and cognition in Tg2576 mice. Therefore, restoring brain tPA levels may be a therapeutic strategy to alleviate the neurovascular and cognitive effects of amyloid pathology.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG071465
NIH Grant R01AG065541
DoD W81XWH-21-10642-01
CIRM Postdoctoral Fellowship EDUC4-12813-01

Title: Shared and distinct the transcriptomic hallmarks of multiple neurodegenerative diseases revealed by short-read snRNA-seq and targeted single nucleus Iso-Seq from human brain.

Authors: *C. PARK[1], C. S. LIU[1], T. NGO[2], J. SAIKUMAR[2], C. PALMER[1], I. COSTANTINO[1], W. W. SEELEY[3], J. CHUN[2];
[1]Univ. of California San Diego, La Jolla, CA; [2]Sanford Burnham Prebys, La Jolla, CA; [3]Neurol., Univ. of California San Francisco, San Mateo, CA

Abstract: Mechanistic understanding of progressive neurodegenerative diseases remains unknown. Neuropathological comorbidities amongst various neurodegenerative disease are commonly observed yet it is unclear whether these diseases share transcriptional hallmarks, particularly in a cell-type-specific manner. We profiled the transcriptome of the human frontal cortex from six different neurodegenerative diseases: Alzheimer's disease (AD, n=6), Lewy body disease (clinically dementia with Lewy bodies (LBD-DLB, n=6)), Lewy body disease (clinically Parkinson's disease (LBD-PD, n=7)), Pick's disease (PiD, n=5), progressive supranuclear palsy (PSP, n=6), and corticobasal degeneration (CBD, n=6), along with non-diseased cortices (ND, n=7). Using single-nucleus RNA-seq (snRNA-seq), we sequenced a total of 246,577 nuclei and identified shared and distinct differentially expressed genes (DEGs) across these diseases. AD, LBD-PD and LBD-DLB samples shared downregulated genes in both excitatory and inhibitory neurons. By contrast, PiD samples showed the highest number of unique DEGs in microglia, compared to the other diseases. Furthermore, we characterized single-nucleus isoform expression and diversity of select genes in each neurodegenerative disease. To determine each isoform's cellular origin, we performed targeted single-nucleus Iso-Seq from the identical library pool as our short-read library prior to fragmentation on 21 selected samples (n=3 per disease). Using target gene enrichment, we interrogated 50 genes based on their expression pattern from our snRNA-seq data across disease and cell type, known isoform diversity, or relevancy to brain diseases and proteinopathies, (*e.g.*, *APP*, *MAPT*, and *SNCA)*. Preliminary results suggest the existence of novel and prevalent isoforms across most targeted genes, including genes with few isoforms in the reference annotation. Ongoing Iso-Seq analyses will be presented on differentially expressed isoforms (DEIs) and the cellular origin of novel isoforms within each condition, providing new insights into human neurodegenerative diseases.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DNAA (NIAAA): T32 AA 025606
DEARC: P50 AA017823/S1

Title: Characterizing gene expression in a rodent model of Alzheimer's disease following binge-like exposure in adolescence

Authors: *P. T. NUNES, N. L. REITZ, K. J. KIRKWOOD, L. M. SAVAGE;
Psychology, SUNY - Binghamton Univ. Behavioral Neurosci., Binghamton, NY

Abstract: Extreme alcohol binge drinking/exposure during early adolescence is a risk of development of neuropsychiatric disorders later in life and is associated with an increased risk for Alzheimer's disease (AD). Both adolescent ethanol exposure and AD cause persistent cognitive dysfunction, deficits in learning and memory, reduction of hippocampal neurogenesis and progressive neurodegeneration of cholinergic neurons in the basal forebrain. Using a model of adolescent intermittent ethanol exposure (AIE) and a transgenic model of AD, male and female TgF344-AD rats (*APP* and *PSEN1* mutations) and wild-type (WT) were exposed to binge-like levels of ethanol or water (postnatal days [PD] 28 to 58). Seven months after AIE (PD305), we investigate the genes expression of AIE and AD-related neuropathologies markers in regions vulnerable to AIE and AD (dorsal hippocampus [dHPC] and medial septum and diagonal band of Broca [MS/DB]). Using real time RT-PCR assessments, we observed sex differences in the fluctuations of housekeeping genes such as RLS, GAPDH and β-Actin in the dHPC. Thus, genes of interest (APP, MAPT, Psen1, Psen2, ChAT, IL-6, vAChT) were normalized to the expression of the housekeeping gene β-Actin in both males and females separately. In the dHPC, expressions of genes of interest were not changed by AIE in either sex. However, a significant genotyping effect was found, where AD animals have significantly higher levels of APP (3-fold increase) and Psen1 (12-fold increase) genes in the dHPC of both sexes compared to WT animals. Selectively in AD male rats, the expression of vAChT in the dHPC and ChAT in the MSDB were increased, however, overall fold-increases were comparatively small relative to APP and Psen1. These results suggest that there is a sexual dimorphism in gene expression, but the reasons are not clear. The lack of exposure effect on the levels of gene expression could be due to the assessment being performed 7 months after the ethanol exposure. Thus, changes in the protein expression of the AIE and AD-related neuropathologies markers will be explored in the hippocampus and entorhinal cortex.

Disclosures: P.T. Nunes: None. **N.L. Reitz:** None. **K.J. Kirkwood:** None. **L.M. Savage:** None.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cell-type specific protein changes in an acute model of Alzheimer's disease

Authors: *J. SHIN[1,2], V. L. DAWSON[1,2,3,4], T. M. DAWSON[1,2,3,5,6], C. NA[1,2], T.-I. KAM[1,2]; [1]Neurodegeneration and Stem Cell Programs, Inst. for Cell Engin., [2]Dept. of Neurol., [3]Solomon H. Snyder Dept. of Neurosci., [4]Dept. of Physiol., [5]Dept. of Pathology, [6]Dept. of Pharmacol. and Mol. Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder. Amyloid-β (Aβ) is known as a central hallmark of AD brains, but the underlying pathological mechanisms still remain to be elucidated. The focus of the majority of AD research has been targeted towards the selective loss of specific neuronal populations, but less effort has been spent in understanding the non-neuronal mechanisms mediated by microglia and astrocytes. While knowledge of proteomic changes that occur in pathological conditions might provide a better understanding of AD pathogenesis, systemic analysis of the brain proteome of AD models is lacking. Here we analyzed the alterations in cell-type-specific proteome induced by Aβ in primary mouse cortical, microglial and astrocyte cultures using TurboID-based protein labeling approach. In addition, lentiviruses expressing TurboID under neuron-, astrocyte, or microglia-specific promoters were delivered to mouse brains via stereotaxic injections, followed by intracerebroventricular injection of oligomeric Aβ that acutely induce AD-like cognitive symptoms. LC-MS/MS-based proteomics in each cell type of Aβ-infused mice brains and further validation via biochemical analysis can offer novel insights into cellular mechanisms underlying AD.

Disclosures: J. Shin: None. **V.L. Dawson:** None. **T.M. Dawson:** None. **C. Na:** None. **T. Kam:** None.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Sex-specific gene expression observed in human dementia with Lewy bodies (DLB)

Authors: *K. C. OLNEY[1], B. E. RABICHOW[2], O. A. ROSS[3], R. CHANG[4], J. D. FRYER[1]; [1]Neurosci., [2]Grad. Sch. of Biomed. Sci., Mayo Clin., Scottsdale, AZ; [3]Neurosci., Mayo Clin., Jacksonville, FL; [4]Biochem., Univ. of Arizona, Tucson, AZ

Abstract: The susceptibility of many neurological diseases varies based on an individual's genetic sex. For example, nearly two-thirds of Americans living with Alzheimer's disease are female, and it has been suggested that genetic females (46, XX) may develop more amyloid plaques due to mounting a more robust innate and adaptive immune response compared to genetic males (46, XY). On the other hand, genetic males (46, XY) are nearly 1.5X more likely to develop Parkinson's disease, characterized by the degeneration of neurons and the deposition of α-synuclein. Little has been reported on sex differences in dementia with Lewy bodies (DLB), which is characterized by α-synuclein protein accumulation in the form of Lewy bodies as well

as the formation of amyloid in the brain. Here we characterize sex differences in gene expression of 307 male and 146 female individuals diagnosed with DLB to determine if the molecular pathways contributing to this disease are shared between the sexes. At the time of abstract submission, our preliminary analysis compares gene expression differences between 58 DLB and 23 age-matched control brains of both sexes. Our results show that genes up-regulated in DLB brains compared to controls when including both sexes are enriched in pathways such as nervous system development, synaptic signaling, and glutamate receptor binding, while down-regulated pathways include oxygen transport and cytoplasmic translocation. When examining each sex separately, we identify that nearly all of the differentially expressed genes observed when utilizing samples from both sexes are driven mainly by the male XY samples, though at this stage this could be due to sample sizes rather than true differences arising due to biological sex. We are analyzing this RNAseq dataset and including important covariates, such as APOE genotype, pathological burden, and comparison to other pathological groups. These studies will further identify the mechanistic pathways contributing to sex differences in neurological susceptibility and outcomes of dementia with Lewy bodies.

Disclosures: K.C. Olney: None. **B.E. Rabichow:** None. **O.A. Ross:** None. **R. Chang:** None. **J.D. Fryer:** None.

Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG072599
AG074004
AG014449

Title: Impact of sex differences on the trajectory of interactome dysfunctions across the Alzheimer's disease spectrum via epichaperomics

Authors: *S. D. GINSBERG[1,2,3,4], T. A. NEUBERT[5], H. ERDJUMENT-BROMAGE[5], M. J. ALLDRED[1,2], A. LABUZA[1,2], A. R. SANTHASEELA[6], A. ALAM[6], S. BAY[6], A. RODINA[6], S. SHARMA[6], C. S. DIGWAL[6], T. WANG[6], G. CHIOSIS[6,7];
[1]Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY; [2]Psychiatry, [3]Neurosci. & Physiol., [4]NYU Neurosci. Inst., [5]Cell Biol., New York Univ. Grossman Sch. of Med., New York, NY; [6]Program in Chem. Biol., Sloan Kettering Inst., New York, NY; [7]Med., Mem. Sloan Kettering Cancer Ctr., New York, NY

Abstract: The effect of sex differences in Alzheimer's disease (AD) remains poorly understood, especially in the context of protein-protein interactions within vulnerable regions that drive dysfunction. Despite growing appreciation of the clinical course, presentation, and severity of

AD dementia, studies of sex impacting AD development and progression are lacking. Although recent high-throughput approaches and bioinformatics technologies help to understand molecular and genetic basis of sex differences in aging and AD, reliance on static 'omics data representing a descriptive inventory of biomolecules measuring changes in their stoichiometry at a given time and condition limits functional insights. Another roadblock is that translating these complex datasets into biological insights requires sophisticated computational algorithms, diminishing access and impact to the biomedical community. To address these limitations, we introduce epichaperomics, an unbiased state-of-the-art high throughput approach which generates direct access to interactome perturbations and to the functional outcome in native biological systems. Epichaperomics is an 'omics method that uses epichaperomes as baits to analyze context-specific alterations in protein connectivity and study disease specific interactomes. Epichaperomics provides direct information on interactome network changes {i.e., what are the global changes in protein-protein interactions (PPIs)} and informs the functional outcomes of such interactome changes (i.e., how are PPI changes executed and what is their negative functional impact). To evaluate feasibility of identifying sex-dependent mechanisms of vulnerability through epichaperomics across the AD spectrum, preliminary studies employed human postmortem AD brain samples (n=8) females and (n=6) males from the prefrontal cortex (BA9). We first confirmed all these AD brain samples expressed epichaperomes, albeit at various levels. Importantly, we found carriers of the ApoE e4 allele were more likely to be high/medium epichaperome expressors, whereas AD subjects with an ApoE3/3 genotype were low epichaperome expressors, suggesting that higher interactome dysfunctions characterize e4 allele carriers, which will now be followed up in a full cohort of female and male subjects from the AD spectrum. We posit a whole new treatment paradigm avenue will open, providing a previously unavailable sex-specific precision medicine approach by understanding and targeting the interactome across the AD spectrum of no cognitive impairment, mild cognitive impairment, and AD dementia through stressor and vulnerability analysis.

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Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1AG062110
NIH AG062677

Title: Post-mortem interval alters transcriptional signatures in the brains of wild-type and tau mice

Authors: *M. P. CADIZ, K. C. OLNEY, K. T. TODD, K. A. HAUG, J. D. FRYER;
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Abstract: Frozen banked brain samples are a valuable resource for the study of neurodegenerative disease. However, the post-mortem interval (PMI), or time between death and frozen preservation of the brain introduces confounds into analysis of banked tissue, especially because PMI varies widely both across and within brain banks. We used single-cell (scRNAseq) and single-nucleus RNA sequencing (snRNAseq) to transcriptionally profile the brains of female wild-type and the PS19 tauopathy mouse model (MAPT-P301S) on a C57BL/6J background immediately after death and three hours after death, to determine how post-mortem interval modifies transcriptional signatures. At three hours PMI, major cell populations in the brain (neurons, astrocytes, microglia, oligodendrocytes, and oligodendrocyte precursor cells) remain identifiable by canonical cell markers for both scRNAseq and snRNAseq. However, a three-hour PMI modifies the transcriptional signatures of several of these subpopulations, including microglia, astrocytes, and oligodendrocytes. At three hours post-mortem, genes associated with heat shock (*Hspa1a*, *Hspa1b*), immune response (*S100a9, S100a9, Lyz2*), and microglial activation (*Apoe, Cst7, Cd74*) are upregulated, while oligodendrocyte and myelin-related genes (*Plp1*, *Mbp, Ptgds*) are downregulated. Furthermore, numbers of both astrocytes and oligodendrocytes are underrepresented at three hours post-mortem, suggesting these cell types may be particularly susceptible to post-mortem effects.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG075820

Title: Integrative whole brain and neuron-type-specific proteomics to examine molecular vulnerabilities of PV interneurons

Authors: P. KUMAR, A. GOETTEMOELLER, S. RAYAPROLU, A. MCWHORTER, J. SANTIAGO, L. CHENG, H. XIAO, D. DUONG, A. SHANTARAMAN, A. NATU, N. T. SEYFRIED, S. RANGARAJU, M. J. ROWAN;
Emory university, Atlanta, GA

Abstract: Vulnerability of Parvalbumin-expressing interneurons (PV-INs) has been observed in several neurological diseases, including Alzheimer's (AD). However, the molecular basis for PV-INs dysfunction is not well understood. Compared to transcriptomics, proteomic investigation of PV-INs can yield insight more proximate to biological function. However, isolating fully intact neurons from adult brain for proteomic studies is challenging. Here we utilize a novel method of cell-type-specific *in vivo* biotinylation of proteins (CIBOP) to investigate proteomic signatures of PV-INs in their native state. CIBOP uses Cre/flox genetics to express biotin ligase TurboID in specific cell types. PV-INs-specific proteomic labeling was achieved by retro-orbital injection of a PV-specific AAV targeting vector co-expressing Cre and GFP , followed by prolonged biotin supplementation in drinking water. IHC imaging confirmed PV-IN-specific labeling without glial activation, and targeted patch recordings confirmed no physiological alterations following biotinylation. Biotinylated proteins were enriched from labeled and control mice and analyzed by label-free quantitative mass spectrometry (MS). We found >1,500 proteins enriched in PV-INs, including canonical markers (Kcnc2, Kcnc3, Calr) and 250 novel PV-IN proteins. Compared with a separate, Camk2a neuron-directed CIBOP proteome, our PV-IN proteome was more enriched in mitochondrial, metabolic, ribosomal, and synaptic vesicle proteins, as well as 28 AD-associated proteins, revealing a molecular phenotype of high metabolic activity and protein turnover in PV-INs. We also investigated whole-brain proteomes from a familial AD (5xFAD) model mice and post-mortem human brain proteomes. Quantitative proteomics by MS of wild type mouse cortex revealed a progressive, age-dependent increase in PV-IN-enriched proteins (Pvalb, Kcnc2). Interestingly, this progressive increase was dampened in 5xFAD mice, while more ubiquitous pan-neuronal proteins (NeuN, Syn) were unaffected by age or genotype. Lastly, a strong correlation between myelination and PV-IN proteins was observed in aged wild-type mice and human brains, suggesting functional interactions between oligodendrocytes and PV-INs. Importantly, our PV-IN proteomic studies are fully adaptable for use in different animal models, and will provide further insights into disease-related PV-INs vulnerability with therapeutic potential.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Michael J. Fox Foundation
The Sidell Kagan Foundation

Title: An investigation into transposable elements in the transcriptomes of patients diagnosed with neurodegenerative disease and healthy controls in urine and plasma

Authors: *M. C. HALL, E. HUTCHINS, C. BILAGODY, R. REIMAN, J. ANTONE, A. LOGEMANN, L. COX, D. M. PALOMARES, F. BITZAH-RAY, K. VAN KEUREN-JENSEN; Translational Genomics Res. Inst., Phoenix, AZ

Abstract: In order to detect and treat diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) earlier, researchers have searched for sensitive and specific biomarkers; however, early detection of diseases of the central nervous system requires a way to easily and accessibly obtain meaningful molecular information. Peripheral biofluids, which can often be obtained quickly and with minimal invasiveness, have promising utility for biomarker discovery and understanding disease pathways. Prior research on the connection between neurodegenerative disorders and changes in the transcriptome has been extensive. More recently, researchers have begun to take a closer look at transposable elements (TEs). TEs are colloquially known as the jumping gene because they move around on the genome. This movement can change gene expression and have implications for disease pathways. The goal of this project was to investigate the role of TEs in plasma and urine from participants with AD, PD or healthy controls. In addition, we wanted to assess the reproducibility of RNA detection and expression levels across time points. Plasma and urine from two visits approximately two-three weeks apart were obtained from participants, as well as corresponding clinical data. Vesicular RNA was isolated from 100 plasma samples (n = 30 AD, n = 40 healthy controls, n = 30 PD) and 90 urine samples (n = 28 AD, n = 36 healthy controls, n = 26 PD). Paired-end, strand-specific libraries were created for sequencing with a targeted sequencing depth of 50 million read pairs per sample. TEs were characterized and quantified using the TEToolkit suite, and differentially expressed TEs were identified using DESeq2. The highest percentage of transposable elements that mapped to the transcriptome were mainly LINE, SINE, and Alu. We found that a number of TEs were differentially expressed in plasma, especially when comparing those diagnosed with AD to healthy controls. Overall, TEs and genes made up a relatively equal proportion of transcripts that map uniquely to the transcriptome across both biofluids, and urine seems to have a slightly higher percentage of uniquely mapping TEs compared to genes. Transcriptomic profiles of TEs will be presented across neurodegenerative diseases and healthy controls, and across plasma and urine biofluids.

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Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARUK-PG2019B-018
The Cure Alzheimer's Fund

Title: Knock-in mouse model, App$^{NLF/NLF}$, with slow plaque development into old age, shows plaque specific gene expression pattern more similar to that found in post-mortem tissues from people with Alzheimer's disease.

Authors: *A. BALBAA, J. WOOD, A. PATHAK, E. WONG, R. JOGHEE, S. DESAI, D. M. CUMMINGS, T. TRIPATHI, J. HARDY, F. A. EDWARDS;
Univ. Col. London, London, United Kingdom

Abstract: Alzheimer's disease (AD) research needs improved mouse models. For reasons of time and cost, most mouse models are designed for rapid plaque deposition, early in life but this could have different effects to slow plaque deposition in old age as happens in the human condition. We compare the gene expression changes in aged *App$^{NLGF/NLGF}$* (NLGF, plaques developing rapidly from 2 months of age) to *App$^{NLF/NLF}$* (NLF, slow plaque deposition from initial detection at 9 months, Saito et al., 2014). Differential expression analysis using RNAseq (n=9-12/genotype; mixed sex; 18 months old) of bulk hippocampal tissue from NLGF or NLF mice compared to wild type (WT) resulted in 2410 differentially expressed genes (DEGs) for NLGF but only 12 DEGs for NLF. This low whole tissue variation in NLF is consistent with the lower plaque load but likely misses changes in different cell types in the immediate vicinity of plaques. We ask whether the differential changes in NLGF represent what would occur in NLF if plaques were more densely present. We thus investigate the genetic consequences of the slowly formed plaques using Nanostring GeoMx technology for cell-enriched spatial transcriptomics (microglia, astrocytes and underlying synapses) in hippocampus of 18 month old NLF mice comparing regions with dense plaques to regions away from plaques (n=6, 4 female, 2 male). No DEGs were detected between WT and the NLF regions away from plaques and so we could avoid cross animal comparisons by pairing plaque versus away regions from the same mouse. Differential genome-wide analysis of NLF, plaque versus away regions resulted in 557 DEGs. While well-known plaque-enriched genes such as *Trem2* and *C1qa* were common to RNASeq in NLGF, 382 of the genes detected were exclusive to NLF spatial analysis and not seen in the whole tissue analysis of the fast plaque forming NLGF model. Functional enrichment analysis of the exclusive genes showed significant representation of cytochrome-c oxidase, Ca^{2+} binding and glutamate transport and release. Furthermore, at least 28 of the exclusive genes are differentially expressed in human AD *postmortem* tissues (Li & De Muynck, 2021), confirming similarity to the human condition. For validation, we first tested plaque specific protein expression of some common genes such as *Trem2* and *Apoe*. Immunohistochemistry showed significant upregulation of these proteins in the plaque region. Validation continues of the exclusive genes. In conclusion, NLF mice with plaque deposition from middle to old age has AD pathology and plaque-associated gene expression closer to human AD than other models. Thus results from this aged model would be more likely to translate to the human condition.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG065541-02
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NIH R01 AG065541-03S1
NIH T32 GM007198-42S1

Title: Evidence for endogenous reverse transcriptase activity in the normal and diseased human brain

Authors: *J. NICODEMUS[1], V. TAN[2], L. RANSOM[1], W. ROMANOW[2], J. CHUN[2];
[1]Univ. of California San Diego, La Jolla, CA; [2]Sanford Burnham Prebys Med. Discovery Inst., La Jolla, CA

Abstract: Somatic gene recombination (SGR) in neurons is a novel neurobiological mechanism that may increase gene copy number and diversity in the normal and diseased brain. The proposed mechanism of SGR requires gene transcription, reverse transcription by endogenous reverse transcriptases (RTs), and DNA strand breaks to allow retro-insertion. This scenario requires the presence of RT activity. However, evidence for endogenous RT activity in the normal or diseased human brain is generally lacking.
We profiled RT activity from neuroanatomically identified brain regions from Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and non-diseased (ND) post-mortem samples using a modified fluorescent product enhanced RT (FPERT) assay. Quantifiable levels of RT activity were identified in the human brain and decreased with increasing age. Furthermore, endogenous RT activity was significantly decreased in the medial temporal cortex of advanced AD brains compared to ND controls, suggesting a potential relationship between disease progression and endogenous RT activity. RT activity was not significantly different between the prefrontal cortex and primary motor cortex of ALS brains and ND brains - notable given the significantly increased endogenous RT activity previously reported in the serum of younger, living ALS patients. The RT activity in the human brain also has a stereotyped sensitivity to various nucleoside and non-nucleoside RT inhibitors that is distinct from HIV RT.
RT activity is present in the human brain and may be modifiable considering the effects of age. Elucidating the range of RT activities in the brain and their relationship to disease may help us better understand its functions in SGR and contributions to disease pathology. RT inhibitors have entered clinical trials for both AD and ALS. The pharmacologically distinct RTi profile of endogenous RT activity may aid identification of the enzyme(s) responsible for RT activity in the brain and in designing potential therapeutics.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.11

Topic: C.02. Alzheimer's Disease and Other Dementias

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W81XWH1910309
10x Genomics 2021 Neuroscience Challenge award

Title: Spatially resolved transcriptomics reveals gene signatures underlying the vulnerability of human middle temporal gyrus in Alzheimer's disease

Authors: *S. CHEN[1], Y. CHANG[1], L. LI[1], D. ACOSTA[1], Y. LI[1], Q. GUO[1], C. WANG[1], C. MORRISON[1], D. JULIAN[2], M. HESTER[2], D. SCHARRE[1], C. SANTISKULVONG[3], S. SONG[3], J. PLUMMER[3], G. E. SERRANO[4], T. G. BEACH[5], Q. MA[1], H. FU[1];
[1]The Ohio State Univ., Columbus, OH; [2]Inst. for Genomic Med., Res. Inst. at Nationwide Children's Hosp., Columbus, OH; [3]Cedars-Sinai Med. Ctr., Los Angeles, CA; [4]Brain and Body Donation Program, [5]Banner Sun Hlth. Res. Inst., Sun City, AZ

Abstract: Background: Recently, spatially resolved transcriptomics (ST) has been used in AD-like mouse models to identify differentially expressed genes (DEGs) in the vicinity of amyloid plaques. Compared with the single-cell or single-nucleus RNA-Sequencing (RNA-Seq) techniques, the recent advent of ST (e.g., 10x Genomics Visium platform) allows us to compare transcriptomic profiles between AD and control subjects without the loss of spatial information. Comprehensive ST profiling of human AD brain tissue and characterization of layer specific DEGs associated with variations of AD pathology, however, have not been reported as far as we know. **Methods:** Human MTG frozen sections from 3 non-AD and 3 early AD cases were mounted over the capture areas with poly T probes on the 10x Visium Gene Expression slide, and the cDNA libraries were generated. Then the cDNA libraries were pooled and sequenced using Illumina NovaSeq6000 sequencer. The sequence data were analyzed via 10x Genomics software (Space Ranger v 1.2.2 and Loupe Browser v 5.0.1) and Seurat (v 4.0.5). Two adjacent (10 μm) sections of the gene expression section were stained with Aβ and AT8 to define Visium spots covering Aβ and/or tau pathology. **Results:** We identify unique marker genes for cortical layers and the white matter, and layer-specific differentially expressed genes (DEGs) in human AD compared to CT. Deconvolution of the Visium spots showcases the significant difference in particular cell types among cortical layers and the white matter. Gene co-expression analyses reveal eight gene modules, four of which have significantly altered co-expression patterns in the presence of AD pathology. We further identify DEGs associated with AD pathology and validate

14 genes using single-molecule fluorescent in situ hybridization. We not only validated some of the previously identified Aβ plaques and tangle-
associated gene signatures and pathways, but also identified new gene signatures and pathways specific for each AD pathology. Importantly, we are the first to identify three unique genes (e.g. KIF5A, PAQR6 and SLC1A3) that are associated with AD pathology whose layer- and cell-type-specific role are unknown. Further functional studies for those genes are warranted in the future. **Conclusions:** In conclusion, we identify layer specific gene signatures associated with human temporal cortical architecture and AD pathology. Our results contribute to the understanding of the complex architecture and responses to AD pathology of this vulnerable brain region.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1RF1AG071683

Title: Spatially resolved transcriptomic characterization of Trisomy 21 in Alzheimer's Disease

Authors: *S. MORABITO[1], E. E. MIYOSHI[1], E. HEAD[2], V. SWARUP[3];
[2]Pathology & Lab. Med., [3]Neurobio. and Behavior, [1]Univ. of California, Irvine, Irvine, CA

Abstract: Gene regulatory programs are highly disrupted in Alzheimer's Disease (AD) in a manner that is concerted with the development of key pathologies such as amyloid beta plaques. APP, the gene encoding amyloid precursor protein, is located on the 21st chromosome, and thus there is a distinct upregulation of APP in individuals with trisomy 21. Using single cell and spatial transcriptomics, we performed an in depth characterization of the frontal cortex and the posterior cingulate cortex from individuals with early and late-stage AD, Down Syndrome with AD (DSAD), and cognitively normal controls. We systematically identified genes that are up- and down- regulated in glial, neuronal, and vascular cell types in each of our disease conditions, and spatially resolved these gene expression signatures. By overlaying our spatial transcriptomic data with amyloid plaque imaging, we identified expression signatures that are spatially associated with plaque deposition. We found shared gene expression changes between DSAD and late-stage AD in astrocytes and vascular cells, as well as distinct dysregulated signatures in other cell types. Furthermore, cell-cell communication network analysis unraveled the signaling pathways that are disrupted due to amyloid deposition.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG054349

Title: Gene Expression Analysis Across the Lifespan of 5xFAD and C57BL6J Mice; Does it Reveal Insight into LTP Impairment?

Authors: *V. ALIZO VERA[1], E. A. KRAMAR[3], K. N. GREEN[4], V. SWARUP[5], M. A. WOOD[2];
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Abstract: Introduction: Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by progressive and irreversible memory deficits and cognitive decline. It is thought that the observed cognitive decline in AD is due to deterioration and loss of synapse function and structure. Although loss of synaptic function and structure have been linked to the well-known Long-Term Potentiation (LTP) impairment that occurs in the aging brain of AD mice, categorization of genes responsible for the documented LTP decline remain to be elucidated.We set out to characterize specific genetic profiles that correlate with the LTP impairment observed in the late stages of AD. To do so we used bulk-RNA sequencing data collected by the MODEL-AD consortium and LTP data taken from 5xFAD and C57BL6J mice at four different aging points (4, 8, 12, and 18 months). **Methods:** In the interest of identifying families of genes possibly involved in the observed LTP decline of the aging 5xFAD mice model, we applied the WGCNA network methodology. We created a synthetic eigengene, based on previously known synaptic plasticity related genes, to understand the relationship between LTP and the synaptic plasticity module. **Findings:** We have identified a specific group of previously characterized synaptic plasticity genes displaying opposing expression profiles between the 5xFAD and C57BL6J mice models. This trend appears to intensify as the mice age, following a comparable trend as the one observed on LTP recordings. We believe this specific group of synaptic plasticity genes could be key for the proper functioning of LTP, and therefore culprits of the defunct LTP that characterizes the AD brain.

Disclosures: **V. Alizo Vera:** None. **E.A. Kramar:** None. **K.N. Green:** None. **V. Swarup:** None. **M.A. Wood:** None.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Alzheimer's Disease Stratification & Novel Target Identification: Combinatorial Analysis of Patient Genomes

Authors: K. TAYLOR, S. DAS, *S. HOGG;
PrecisionLife, Oxford, United Kingdom

Abstract: Alzheimer's disease (AD), like other complex diseases, is characterised by a high degree of heterogeneity across the patient population, reflected in a wide range of disease presentations and therapy responses. GWAS have identified several disease-associated genes, but these findings have not translated into progress in clinical trials. This probably reflects the fact that GWAS is limited to identifying single variants with large effect sizes in a population, while the key to understanding complex diseases such as AD that are influenced by multiple genetic loci, epidemiological and/or environmental factors is to find combinations of these disease associated factors that distinguish one patient subgroup from another. The PrecisionLife platform utilises a hypothesis-free method for the detection of combinations of features that together are strongly associated with variations in disease risk, symptoms, progression rates and therapy response often observed in AD patient subgroups. To explore the substructures within the AD population we analysed a genomic dataset from the UK Biobank, including 882 patients diagnosed with AD as defined by the ICD-10 code G30.x. These patients were compared against healthy controls with no reported neurodegenerative disorders, self-reported cognitive decline or AD, and no family history of AD. The oldest available controls (to minimize the potential for late onset disease) who met these criteria were gender-matched and selected in a 2:1 ratio against the cases. Our analysis identified combinations of genetic variants which mapped to 113 genes that are significantly associated with AD development. Clustering these combinations, based upon the patients in which they were found, generated six major subgroups of patients. Each of these patient groups reflected a specific biological function - lipid metabolism, neuroinflammation, autophagy, serotonin receptor signaling, metal ion homeostasis, and adipose tissue differentiation/fatty acid synthesis. Furthermore, the specific combinations of variants associated with each patient subgroup serve as a biomarker to identify patients most likely to be responsive to pharmacological modulation of the target or pathways identified. The results demonstrate that the PrecisionLife combinatorial analysis is uniquely able to stratify heterogenous patient populations with complex disease pathologies. We can use these insights to identify more effective therapeutic strategies and accompanying biomarker sets to match them to the patient subgroups that are most likely to demonstrate benefit in downstream clinical trials.

Disclosures: K. Taylor: A. Employment/Salary (full or part-time):; PrecisionLife. **S. Das:** A. Employment/Salary (full or part-time):; PrecisionLife. **S. Hogg:** A. Employment/Salary (full or part-time):; PrecisionLife.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01AG072883

Title: Transcriptome analysis reveals autophagy preservation in cognitive intact individuals with Alzheimer's disease neuropathology

Authors: *P. SCADUTO[1], G. TAGLIALATELA[2];
[1]UTMB, Galveston, TX; [2]Neurol., Univ. of Texas Med. Br. Dept. of Neurol., Galveston, TX

Abstract: Sporadic Alzheimer disease (AD) is by far the most common form of dementia characterized by abnormal accumulation of toxic oligomers that leads to formation of plaques, neurofibrillary tangles, and ultimately neuronal deficit and loss of cognitive ability of the subject. In recent years, several reports have described rare individuals, referred to "Non-Demented with Alzheimer's Neuropathology" (NDAN), showing severe neuropathological signs, though remaining cognitive intact. The existence of these unusual cases suggests that there is a natural way for the human brain to resist (or significantly delay) the neurotoxic events that normally lead to cognitive impairment in AD. We hypothesized that one of those potential protective mechanism could be the enhancing autophagical efficency. Autophagy is an intracellular system designed for the degradation of long-lived proteins and organelles in lysosomes, and it has been suggested that its alterations may be involved in abnormal accumulation of toxic proteins. Autophagy activity naturally reduces with age, that is the major risk factor of AD. In individuals with AD pathology this reduction is exacerbated and significantly reduced compared to non-AD age matched individuals. Our lab analyzed autophagic levels of proteins (Tumurbaatar B. SFN poster) and mRNA (this poster) from hippocampus and cortical areas of two independent cohorts of cognitively normal, AD, NDAN donors. Here, using available online RNA-sequencing dataset we separate CTRL (N=29), AD (N=39), and NDAN (N=22) based on 3 criteria: braak stage, Cerad score and cognitive test. We found strong correlation between expressions of MAPT and several autophagy proteins. Cortical and hippocampal regions from AD subjects showed increased levels of mTOR complex components (mLST8, Raptor and PRAS40) that are known to block the autophagy activity. Interestingly, we found preserved levels of those mRNAs in NDAN donors. In addition, mTOR inhibitor Deptor mRNA was reduced in hippocampus and parietal cortex of NDAN individuals. This evidence suggests that proper levels of key autophagy elements mRNAs as well as of mTOR complex components may contribute to preservation of cognitive integrity in the face of profuse AD neuropathology as observed in NDAN individuals.

Disclosures: P. Scaduto: None. **G. Taglialatela:** None.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

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Program #/Poster #: 613.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIMH U01 MH106892
NIMH U01 MH106893
NIMH U01 MH106882

Title: Mapping the complex genetic landscape of human neurons

Authors: *M. J. MCCONNELL[1], C. SUN[2], K. KATHURIA[1], S. B. EMERY[3], B. KIM[2], I. E. BURBULIS[5], D. R. WEINBERGER[1,6], J. V. MORAN[3,4], J. M. KIDD[2,3], R. E. MILLS[2,3]; [1]Lieber Inst. for Brain Develop., Baltimore, MD; [2]Computat. Med. and Bioinformatics, Univ. of Michigan, Ann Arbor, MI; [3]Human Genet., [4]Intrnl. Med., Univ. of Michigan, Ann Arbor, MI; [5]Univ. San Sebastian, Puerto Montt, Chile; [6]McKusick-Nathans Ctr. for Genomic Med., Johns Hopkins Sch. of Mediine, Baltimore, MD

Abstract: A subset of human neurons acquire apparently idiosyncratic copy number variants (CNVs, CNV neurons) during development. Cross sectional analysis of these neurons indicates that cortical CNV neurons are selectively vulnerable to age-related loss. Most somatic cells that acquire complex karyotypes are removed by the immune system. Mutant somatic cells that escape the immune system often lead to cancer except in the brain. Neurons are almost never the origin of brain cancers. Instead, somatic mutations in neurons contribute to the polygenic landscape of neuropsychiatric and neurodegenerative disease. We developed an allele-based validation approach, SCOVAL, to corroborate or reject read-depth based CNV calls in single human neurons, applied this approach to 2125 frontal cortical neurons, and identified a class of CNV neurons with complex karyotypes containing whole or substantial losses of multiple chromosomes. Moreover, we find that CNV location is non-random, and identify recurrent regions of neuronal genome rearrangement associated with long genes.

Disclosures: M.J. McConnell: None. **C. Sun:** None. **K. Kathuria:** None. **S.B. Emery:** None. **B. Kim:** None. **I.E. Burbulis:** None. **D.R. Weinberger:** None. **J.V. Moran:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent US6150160. F. Consulting Fees (e.g., advisory boards); Gilead Sciences, Tessera Therapeutics. **J.M. Kidd:** None. **R.E. Mills:** None.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG061796

Title: Integrative Multi-Omics Studies to Identify Molecular Pathways of Resilience to Alzheimer's Disease

Authors: *W. TSAI[1], C. E. MCNIFF[1], J. S. REDDY[1], Z. S. QUICKSALL[1], X. WANG[1], M. ALLEN[1], K. KANTARCI[2], J. GRAFF-RADFORD[2], C. R. JACK, Jr.[2], M. M. MIELKE[2], R. C. PETERSEN[2], M. M. CARRASQUILLO[1], N. ERTEKIN-TANER[1];
[1]Mayo Clin., Jacksonville, FL; [2]Mayo Clin., Rochester, MN

Abstract: The goal of this study is to identify genes, variants, and molecular networks that contribute to cognitive resilience in the presence of AD risk factors. To address those, we have generated and integrated whole genome sequencing (WGS, n=143) and blood RNA-sequencing (RNA-seq, n=105) data from individuals at high risk (APOE ε4 risk allele carriers > 80 years of age) but remain cognitively normal. Applying genome-wide association study (GWAS), differential gene expression (DGE) and weighted gene co-expression network (WGCNA) analyses, we tested the association of genetic variants and gene expression with various phenotypes. Phenotypes included diagnosis (resilient vs non-resilient), neuroimaging [amyloid burden via Pittsburg compound B positron emission tomography (PiB PET), white matter hyperintensity (WMH), infarctions, microhemorrhages (MCH)], and cognitive scores [global cognitive, memory, language, attention, visuospatial, logical memory delayed recall (LMDR)]. GWAS detected 761 suggestively significant ($p<1x10^{-5}$) variants associated with diagnosis, PET, WMH, and/or cognitive scores. DGE analysis identified significantly lower expression of *RP11-531F16.4* (beta= -0.76, $q<0.05$) associated with Non-Resilience. No significant associations were detected in DGE analyses of other phenotypes, but genes with suggestive associations ($q<0.1$) were detected with LMDR. WGCNA identified nominally significant associations ($p<0.05$) between modules eigengenes (MEs) and WMH, MCH, memory, language, and/or LMDR. Of all the phenotypes, LMDR had the most significant ME correlations. Interestingly, ME12 was the most significant module correlated with LMDR, and was significantly enriched ($q<0.05$) with biological processes related to epigenetic regulation. Cell-type enrichment analysis revealed that among modules that are significantly correlated with phenotypes, module 2 was enriched (Bonferroni-adjusted $p<0.05$) for red blood cell marker genes and this module was also significantly associated with LMDR. We are performing *cis*-expression quantitative trait locus (*cis*-eQTL) analysis to identify the regulatory impacts of the variants associated with the various resilience/non-resilience phenotypes. Altogether, our study has nominated genes, variants and pathways that may drive cognitive resilience in individuals who are at high risk of developing AD.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Frontier Research unit (6501005490)
KMUTT Research Fund2021
Asahi glass foundation Overseas research grant2020

Title: Transcriptional signature genes associated with synapse, mitochondria, and cytoskeleton of hippocampal and entorhinal cortex neurons in Alzheimer's brain

Authors: *P. NAMCHAIW, S. AIMAUTHON;
King Mongkut's Univ. f Technol. Thonburi, Bangkok, Thailand

Abstract: Alzheimer's disease (AD) is one of the major causes of dementia in the elderly worldwide. However, the causes of the disease are not fully understood. The pathological hallmarks of AD are microtubule destabilization, synaptic degeneration, and mitochondrial dysfunction. The neurons especially in the hippocampus (HP) and entorhinal cortex (EC) are those selectively vulnerable cells and thus affect the learning and memory of patients. Several previous studies had attempted to understand the disease by studying gene expression profiles. In this study, we performed a transcriptional analysis of five datasets from HP tissue (GSE1297, GSE28146, GSE36980, GSE48350, and GSE5281) and two datasets from EC tissues (GSE48350 and GSE5281). We focused on the differential gene expression (DGE) in the structure of synapse, mitochondria, and cytoskeleton. We identified 56 common genes in HP and 83 common genes in EC. Of which, 58 common genes were identified in the synapse, mitochondria, and cytoskeleton cellular compartments. These gene-encoding proteins were mapped to the protein-protein interaction network and identified hub genes using Cytoscape. We identified a subnetwork of 129 nodes and 122 edges, of which 10 genes have been reported to be associated with AD. The largest hub of the protein-protein interaction network was ATP5B followed by UQCRQ. Our study demonstrated the common DEGs in three cellular compartments including synapse, mitochondria and cytoskeleton, and identified the genes which may be important in AD progression.

Disclosures: P. Namchaiw: None. **S. Aimauthon:** None.

Poster

613. Alzheimer Disease Multiomics

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Topic: C.02. Alzheimer's Disease and Other Dementias

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BrightFocus Postdoctoral Fellowship in Alzheimer's disease research
Yale ADRC Research Scholar Award
R01NS115544
R01NS111961
Cure Alzheimer's Fund Research Grant
RF1AG058257

Title: Spatial proteomics and iPSC modeling uncover therapeutic targets for axonal pathology in Alzheimer's disease

Authors: *Y. CAI[1], J. KANYO[2], R. WILSON[2], M. SHAHID MANSURI[2,3], P. L. CARDOZO[3], D. GOSHAY[4], Z. TIAN[1], A. BRAKER[4], K. TRINH[1], T. LAM[2], K. BRENNAND[3,5], A. C. NAIRN[3,6,2], J. GRUTZENDLER[1,7];
[1]Neurol., [2]Yale/NIDA Neuroproteomics Ctr., [3]Psychiatry, [4]Yale Col., [5]Genet., [6]Pharmacol., [7]Neurosci., Yale Univ., New Haven, CT

Abstract: Amyloid deposits in Alzheimer's disease (AD) are surrounded by massive numbers of plaque-associated axonal spheroids (PAAS). PAAS correlate with AD severity and may affect axonal electrical conduction and neuronal network. However, the molecular mechanisms that govern their formation are not well understood. To tackle this, we applied proximity labeling in AD postmortem brains of humans and mice, followed by LC/MS/MS to uncover the PAAS-proteome. We then implemented a human iPSC-derived AD model that recapitulates PAAS pathology, to examine potential roles of proteome hits. Using this strategy, we uncovered hundreds of previously unknown PAAS-enriched proteins and signaling pathways. We found that phosphorylated mTOR was highly enriched in PAAS in postmortem human brains and strongly correlated with disease severity. Pharmacological mTOR inhibition in iPSC-derived human neurons or in vivo AAV-mediated knockdown in mice led to a marked reduction in PAAS pathology. Altogether, we provide novel resources for investigating axonal pathology in AD.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG054345

Title: Transcriptional assessment of novel mouse models harboring human genetic variants associated with late-onset Alzheimer's disease

Authors: G. CARTER[1], C. PREUSS[1], R. PANDEY[1], A. UYAR[3], D. GARCEAU[1], K. P. KOTREDES[2], H. WILLIAMS[1], A. OBLAK[4], P. B.-C. LIN[4], B. PERKINS[4], D. SONI[4], C. INGRAHAM[4], A. LEE-GOSSELIN[5], B. T. LAMB[4], G. R. HOWELL[1], M. SASNER[1]; [2]Res., [1]The Jackson Lab., Bar Harbor, ME; [3]The Jackson Lab. for Genomic Med., Farmington, CT; [4]Stark Neurosciences Res. Inst., Indianapolis, IN; [5]Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Genome-wide association studies have identified over 70 genetic loci robustly associated with late-onset Alzheimer's disease (LOAD), and the associated risk genes suggest a diversity of contributing pathways. Translation of these findings to new treatments requires a deeper mechanistic understanding and appropriate preclinical models for the testing of targeted therapeutics.

We have introduced 12 candidate genetic variants into a sensitized mouse model already harboring humanized APOE4 and Trem2.R47H alleles on a C57BL/6J background. Variants were selected based on predicted function, cross-species conservation, increased risk of LOAD, and allele frequency. Genome editing with CRISPR-Cas9 was performed and mouse cohorts were aged to four, eight, and 12 months. Homogenized brain hemispheres were assayed from both male and female mice with the Nanostring Mouse AD Panel to measure expression of 800 genes selected for human disease relevance.

Transcriptomic effects from multiple genetic variants recapitulated a variety of human gene expression patterns observed in LOAD study cohorts. We observed changes in genes annotated for myelination, metabolism, and extracellular matrix that were not observed in early-onset transgenic 5xFAD mice. Notable examples include effects on protein folding genes in ABCA7.A1527G mice, metabolism genes in MTHFR.677C>T mice, and synaptic vesicle genes in PLCG2.M28L mice. Many late-onset variants exhibited increasing LOAD-like effects with age, supporting the role of these variants in age-related disease. Although baseline sex differences were observed, most strains did not show significant sex-specific effects relevant to LOAD.

We have characterized in vivo signatures of 12 genetic candidates for LOAD, identifying alterations in specific LOAD-related pathways in each variant on a sensitized genetic background. These results provide an initial functionalization of LOAD genetic factors and provide animal models for preclinical testing of therapeutics designed to correct specific molecular alterations that contribute to LOAD pathology and progression.

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Poster

613. Alzheimer Disease Multiomics

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG055104
AG054345

Title: Genetically diverse mouse models of Alzheimer's disease reveal an association between glycan metabolism and cerebral amyloid angiopathy

Authors: *K. J. ELK[1], A. UYAR[2], D. GARCEAU[3], R. O'ROURKE[3], K. P. KOTREDES[4], K. D. ONOS[4], G. W. CARTER[3], G. R. HOWELL[3], M. SASNER[3];
[1]The Jackson Lab., The Jackson Lab., Bar Harbor, ME; [2]The Jackson Lab., Farmington, CT; [4]Res., [3]The Jackson Lab., Bar Harbor, ME

Abstract: A growing body of research is revealing the benefits of incorporating genetically diverse mouse strains to better model Alzheimer's disease (AD). For instance, mouse models of AD on genetically distinct wild-derived mouse strains demonstrated robust differences in immune response to amyloid and neurodegeneration, better representing the phenotypic spectrum of AD than previous models. To further investigate the importance of genetic diversity, we are generating AD-relevant mouse models on at least ten Collaborative Cross (CC) strains, a recombinant inbred mouse panel created from eight highly diverse founder strains. To date, C57BL/6J (B6J) mice homozygous for humanized APOE4 allele and carrying mutant APP and PS1 transgenes (APPswe, PS1de9) have been crossed to five CC strains that were selected to maximize variation in AD-relevant genes, including *Trem2* and *Cd33*. Cohorts of male and female B6JCCnF1.APOE4.APP/PS1 mice and controls (6/sex/strain/Tg-genotype) were aged to 8 months (a midpoint for amyloid deposition in B6.APP/PS1 mice) and brain hemispheres were processed for transcriptional profiling by RNA-seq and neuropathological assessments (including amyloid deposition and neurodegeneration) via immunohistochemistry (IHC). Data were assessed for strain-, genotype- and sex-specific variation. IHC data revealed marked differences in amyloid deposition across the CC strains. For instance, in the presence of the amyloid drivers, B6J/CC037F1 and B6J/CC006F1 exhibited elevated levels of parenchymal plaque compared to B6J. Vascular amyloid, indicative of cerebral amyloid angiopathy (CAA), varied greatly across the strains, independent of parenchymal amyloid load, with B6J/CC037F1 showing the highest levels of CAA as determined by preliminary CAA severity scores. We observed significant CC strain- and sex- specific variation in immune (Fc gamma R-mediated phagocytosis) and metabolic (glycosphingolipid biosynthesis) response to amyloid pathogenesis. Differential expression (DE) and weighted gene coexpression analysis (WGCNA) analyses of RNA-Seq data independently correlated CAA scores with glycosylation/glycan pathways. To further pursue this link, CAA severity scores have been reassessed using guidelines developed for human tissue, as utilized in a recent publication (Reddy, J. S. et. al, 2021). Realignment to RNA-Seq data and subsequent rounds of IHC to investigate a potential association between protein glycosylation and CAA are underway. Overall, this study further supports the incorporation of genetically diverse mouse strains, including CC strains, to better model complex neurodegenerative disorders such as AD.

Disclosures: **K.J. Elk:** None. **A. Uyar:** None. **D. Garceau:** None. **R. O'Rourke:** None. **K.P. Kotredes:** None. **K.D. Onos:** None. **G.W. Carter:** None. **G.R. Howell:** None. **M. Sasner:** None.

Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01 AG065206-01
NIH Grant 1F32 AG069342-01A1
Mathers Charitable Foundation

Title: Changes in neuronal gene expression related to early synaptic phenotypes in Alzheimer's model mice

Authors: *J. TOMORSKY, M. DJURISIC, C. J. SHATZ;
Biol., Stanford Univ., Stanford, CA

Abstract: Synaptic dysfunction occurs early in the pathogenesis of Alzheimer's Disease (AD) and is the strongest correlate of cognitive decline in AD patients. In an APP/PS1 mouse model of AD, synaptic plasticity and ocular dominance plasticity deficits have been documented at postnatal day 30 (P30), long before the appearance of amyloid plaques and immune dysregulation. Here, we investigate potential neuronal mechanisms driving these early plasticity deficits in P30 APP/PS1 mice. A neuron-specific ribosomal tagging approach (Snap25-Cre:RiboTag) was employed to isolate ribosome-bound mRNA from APP/PS1 and control cortical neurons, followed by RNA-seq. Relative to control, neuronal mRNA isolated from P30 APP/PS1 mice reared with normal experience had decreased expression of immediate early genes and genes involved in MAPK signaling, both well known to be involved in plasticity. Manipulating the sensory environment of animals at this age changed the differential neuronal gene expression observed between APP/PS1 and control mRNA, revealing a deficit in mRNA expression related to neuron projection development and synapse organization in APP/PS1 neurons. These early changes in neuronal gene expression in APP/PS1 mice, both at baseline and after sensory perturbation, could contribute to the plasticity deficits previously reported in these mice. Paradoxically, in normally reared P30 APP/PS1 mice, expression of genes associated with synaptic transmission is increased. Recording of AMPAR mEPSCs from L2/3 cortical pyramidal neurons at this age revealed an increase in mEPSC frequency in APP/PS1 mice, congruent with the upregulation of synaptic transmission-associated genes. However, a small, but significant, drop in mEPSC amplitude was also observed, suggesting synapses may be weaker than normal. The changes in plasticity-related gene expression observed here in juvenile APP/PS1 mice, in tandem with synaptic and plasticity defects, suggest an onset of APP-dependent pathology that

is, at least in part, of neuronal etiology and starts well before plaques and cognitive deficits appear.

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Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.23

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of 15 Alzheimer's disease risk genes from UK Biobank 450k individuals' whole exome sequencing data analysis

Authors: *T. MONJO, J. LEE, A. SUGIURA, T. ANDO;
Takeda Pharmaceut. Co., Fujisawa, Kanagawa, Japan

Abstract: Identification of genetically validated novel drug targets for Alzheimer's disease (AD) is extremely important because retrospective studies suggested that genetically supported targets were more likely to be successful in drug development. The partnership between UK Biobank (UKB) and pharmaceutical companies completed the sequencing of whole exome for almost all UKB participants. Whole exome sequencing (WES) enables us to detect rare deleterious variants related to various diseases. To identify potential risk genes to AD, we conducted association tests on AD and family history of AD using 450k individuals' WES data in UKB. To be specific, we firstly defined proxy AD patients from family history of AD using statistic models (e.g. LTFH model), and conducted gene-based and variant-based association tests of rare loss-of-function and predicted deleterious missense variants (minor allele frequency < 1%) using scalable linear-mixed model (i.e. SAIGE-GENE) to increase statistical power. As a result, we found 15 genes that were significantly associated to AD. 7 of the genes tended to be associated with AD by direct case-control study, and 8 of the genes were supported by public GWAS/EWAS. In conclusion, we identified the potential genetic associations including novel genes associated with AD. Further replication and wet validation study would prioritize these gene candidates for potential drug development programs by generating and supporting therapeutic hypothesis of AD.

Disclosures: T. Monjo: A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **J. Lee:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **A. Sugiura:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **T. Ando:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company.

Poster

613. Alzheimer Disease Multiomics

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Program #/Poster #: 613.24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG054223
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NIH Grant S10OD030346

Title: Characterization of region-specific neuronal BIN1 function in tau pathogenesis by spatial whole transcriptomics analysis in tau transgenic mice

Authors: *S. WANG, G. THINAKARAN, M. PONNUSAMY;
Dept. of Mol. Med., USF Morsani Col. of Med., Tampa, FL

Abstract: Bridging integrator 1 (BIN1), a late-onset Alzheimer's disease (AD) risk factor identified via genome-wide association studies, encodes an adaptor protein that regulates membrane remodeling and membrane dynamics in the context of endocytosis and synaptic vesicle release. BIN1 can directly bind to tau, leading to the key hypothesis for the role of BIN1 in AD in the aggravation of tau pathology. Preliminary characterization of tau pathogenesis in *Emx*-Cre *Bin1*-cKO mice reveals a complex picture: the loss of forebrain BIN1 expression in P301S tau transgenic mice (line PS19) exacerbated tau pathology in the somatosensory cortex, thalamus, spinal cord, and sciatic nerve, accelerated disease progression, and caused early death. Intriguingly, the loss of BIN1 also mitigated tau neuropathology in select regions, including the hippocampus, entorhinal/piriform cortex, and amygdala, thus attenuating hippocampal synapse loss, neuronal death, neuroinflammation, and brain atrophy. RNAseq analyses revealed that the loss of forebrain BIN1 expression elicited complex neuronal and non-neuronal transcriptomic changes, including altered neuroinflammatory gene expression. We are performing spatial transcriptomics using the GeoMX DSP system to understand how neuronal BIN1 function contributes to spatiotemporal heterogeneity in tau pathophysiology. Spatial transcriptomics technology will allow us to identify the region-specific manifestation of tau pathology-related neuronal and synapse loss and neuroinflammatory changes in PS19 mice expressing or lacking BIN1 in forebrain excitatory neurons. We have performed a longitudinal analysis of PS19:*Emx*-Cre:*Bin1*-cKO and mice PS19:*Emx-Cre* controls to characterize BIN1 expression-related molecular pathways and key drivers of disease that reflect changes that occur early- or late in tau pathogenesis. Our findings will highlight exciting region-specificity in BIN1 regulation of tau pathogenesis.

Disclosures: S. Wang: None. **G. Thinakaran:** None. **M. Ponnusamy:** None.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant MH109260
NIH Grant T32 MH014654
Pennsylvania Department of Health Tobacco Settlement Act Grant RFA67-76
2017 NARSAD Young Investigator Grant #26634

Title: Differential gene expression and transcript usage in mouse models implicates cell types in Alzheimer's disease pathology

Authors: *A. WELLER[1], T. FERRARO[2], G. DOYLE[1], B. REINER[1], R. CRIST[1], W. BERRETTINI[1];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia and is a source of significant morbidity and mortality for patients and their families. However, the specific cause of AD remains poorly understood. 5XFAD humanized mutant mice and Trem2 knockout (T2KO) mice are two mouse models relevant to the study of AD-related pathology. The aim of this study was to determine hippocampal transcriptomic and polyadenylation site usage alterations caused by genetic mutations engineered in 5XFAD and T2KO mice. Employing a publicly available single-nucleus RNA sequencing dataset, we used Seurat and Sierra to identify differentially expressed genes (DEGs) and differential transcript usage (DTU), respectively, in hippocampal cell types from each of the two mouse models. We analyzed cell type-specific DEGs further using Ingenuity Pathway Analysis (IPA). We identified several DEGs in both neuronal and glial cell subtypes in comparisons of wild type (WT) vs. 5XFAD and WT vs. T2KO mice, namely *Ttr*, *Fth1*, *Pcsk1n*, *Malat1*, *Rpl37*, *Rtn1*, *Sepw1*, *Uba52*, *Mbp*, *Arl6ip5*, *Gm26917*, *Vwa1*, and *Pgrmc1*. We also observed DTU in common between the two comparisons in neuronal and glial subtypes, specifically in the genes *Prnp*, *Rbm4b*, *Pnisr*, *Opcml*, *Cpne7*, *Adgrb1*, *Gabarapl2*, *Ubb*, *Ndfip1*, *Car11*, and *Stmn4*. IPA identified 3 statistically significant canonical pathways that appeared in multiple cell types and that overlapped between 5XFAD and T2KO comparisons to WT, specifically 'FXR/RXR Activation', 'LXR/RXR Activation', and 'Acute Phase Response Signaling'. DEG, DTU, and IPA findings, derived from two different mouse models of AD, highlight the importance of energy imbalance and inflammatory processes in specific hippocampal cell types, including subtypes of neurons and glial cells, in the development of AD-related pathology. Additional studies are needed to further characterize these findings.

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Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.26

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Empirical Validation of Genetic Imputations with Whole Genome Data Reveals Sex Differences in Alzheimer's Disease Polygenic Risk Score Analyses

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Abstract: Recent polygenic risk scores (PRS) for late-onset Alzheimer's disease (LOAD) have reached inconsistent conclusions concerning whether the disease exhibits an oligogenic or polygenic pattern of inheritance, differing in the amount of phenotypic variance or heritability (h^2) they explain, and how much the rest of the genome beyond APOE, the major risk gene for LOAD, contribute to disease risk. The primary methodological differences between these studies appear to be in the genetic imputation strategy used in their validation set. In addition, it has long been known that the APOE4 allele exerts a stronger effect on disease risk in females than in males, but it is currently unknown if the rest of the genome carries a similar sex-dependent risk. To identify methodological differences yielding such divergent conclusions and explore the power of a genome-wide sex-specific vs sex-agnostic PRSs, we extracted subjects of Caucasian ancestry from the NIAGADS and ADNI databases (N=25,106), and imputed their data using the University of Michigan Imputation Server (UMIS) to produce a larger set of overlapping genotypes. A second set of 1,204 subjects, composed of ADNI 2/GO and 1000 Genomes Caucasian ancestry subjects with Whole Genome Sequencing (WGS), were compiled for our Imputation Validation dataset. This data was used to extract the set of pre-imputation single nucleotide polymorphisms (SNPs) submitted from the imputed cohorts, and likewise run through the UMIS. We compared the set of post-imputation SNPs with the known SNPs from the WGS data and calculated the Matthews Correlation Coefficient (MCC) to estimate their empirical accuracy. The standard imputation quality measure (Info-Score or R^2) and our empirical validation measure (MCC^2 to place it on the same scale as the Info-Score) were used to separately extract two datasets that were quality controlled identically.
In the PRSs calculated from the SNPs selected by Info-Score imputation quality, we found the best h^2 estimates being derived from a small set of SNPs (supporting an oligogenic model) usually with the smallest p-value threshold and equivalent to the predicted h^2 of using the APOE4 allele alone. Using empirically validated SNPs, however, we observed a pattern of polygenic inheritance: the best h^2 estimates derived from a high p-value threshold including nearly the entire set of SNPs, surpassing the observed h^2 for APOE4 alone.
In the empirically validated derived sets, the sex-specific PRS models produced higher observed h^2 than the sex-agnostic models, and in particular, the heritability in women was higher than in men, perhaps indicating that genetics may play a larger role in AD development for females.

Disclosures: C. Patterson: None. **S. Andrews:** None. **J. Pa:** None. **P.M. Thompson:** None.

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

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Program #/Poster #: 613.27

Title: WITHDRAWN

Poster

613. Alzheimer Disease Multiomics

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 613.28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG071465
CIRM Postdoctoral Fellowship (EDUC4-12813-01)

Title: Unique cell-type-specific RNA isoforms in Alzheimer's disease brain cells revealed by single-nucleus short and long-read sequencing

Authors: *T. NGO[1], C. S. LIU[1,2], C. PARK[1,2], J. SAIKUMAR[1], C. R. PALMER[1,2], J. CHUN[1]; [1]Translational Neurosci., Sanford Burnham Prebys, La Jolla, CA; [2]Univ. of California San Diego, La Jolla, CA

Abstract: The mechanism of Alzheimer's disease (AD) progression remains uncertain, while disease-modifying therapies are nonexistent. Studies exist exploring the molecular complexity of AD in relevant brain regions using short-read single-nucleus RNA-seq (snRNA-seq), which enables the delineation of cell-type gene expression changes within a heterogeneous tissue sample on a single cell level. These studies have also revealed potential therapeutic targets for AD. However, one inherent limitation of most short-read sequencing is the inability to capture RNA isoforms, representing an additional layer of consideration for understanding biology and identifying drug targets with therapeutic potential. We hypothesized that single-nucleus long-read isoform sequencing would reveal cell-type-specific RNA isoforms that are uniquely expressed in AD compared to non-diseased samples. Here, we performed short-read snRNA-seq (n=6-7) coupled with single-nucleus long-read isoform sequencing (n=3) on the same barcoded cDNA libraries of AD and non-diseased human prefrontal cortex samples. Cell types in each sample were readily resolved. In contrast to a previously published short-read snRNA-seq AD dataset from human prefrontal cortex tissue, dysregulation of genes was observed evenly across all cell types, including excitatory neurons, astrocytes and microglia. Moreover, our data is consistent with microglia activation in AD, with reduced expression of critical homeostatic markers. Long-read isoform sequencing revealed vast RNA isoform diversity in all samples, including novel transcripts not categorized in reference databases. Importantly, transcripts were

matched to cell type, as annotated by short-read sequencing, to uncover cell-type-specific RNA isoforms in AD. Ongoing analysis includes assessing the biological importance of dysregulated genes and their novel isoforms, which may represent unique therapeutic targets expressed in AD. Overall, the expansion of the transcriptome via long-read sequencing will shed new insight into AD pathology.

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Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 614.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC
FRQS
HBHL
Weston

Title: Multimodal assessment of white matter hyperintensity severity

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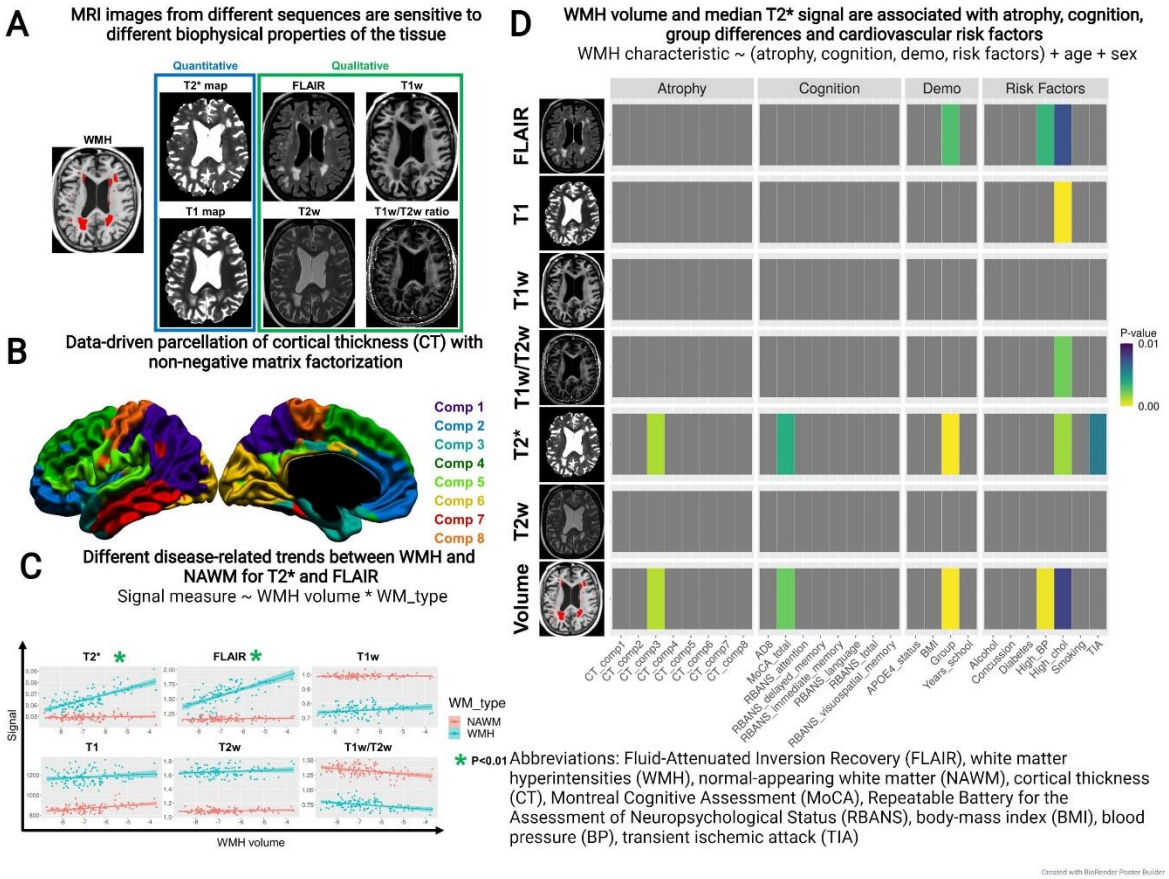
Abstract: White matter hyperintensities (WMH) detected on magnetic resonance imaging (MRI) are commonly found in elderly populations and are often associated with neurodegeneration, cardiovascular risk factors, and adverse cognitive outcomes. However, the WMH volume does not capture the heterogeneous microstructural substrates of WMHs. We explore if different biophysical properties of WMH tissue, assessed with different MRI images, are sensitive to cognitive and clinical variation across the Alzheimer's Disease (AD) spectrum.
We used multiple MRI images (T1w, T2w, T1w/T2w ratio, FLAIR, quantitative T1, quantitative T2*) (Fig 1A), demographic, cognitive, and cardiovascular risk factors variables that were acquired on 118 elderly participants categorized as cognitively healthy, at high-risk for AD

(familial history), mild cognitive impairment, or AD. WMHs were automatically segmented and WMH volumes were divided by total brain volume and log-transformed. The median MRI signal inside WMHs and the normal-appearing white matter (NAWM) was calculated. Cortical Thickness (CT) was estimated with CIVET and parcellated in a data-driven fashion with non-negative matrix factorization (Fig 1B).

The interaction between WMH volume and white matter type (WMH or NAWM) only yielded differences in the T2* and FLAIR measures, potentially indicating that these measures are sensitive to microstructural alterations that are more specific to WMH (Fig 1C). In univariate analyses controlling for age and sex, our results included significant associations at $p<0.01$ between WMH T2* signal and medial temporal lobe CT, Montreal Cognitive Assessment scores, group differences, high cholesterol, and transient ischemic attacks (Fig 1D).

WMH T2* signal could be sensitive to a clinically-relevant degenerative process of WMH that is different from NAWM microstructural alterations. We speculate that the increased T2* in WMH represents a higher degree of inflammation and demyelination. Other signal measures, except FLAIR, could be most sensitive to the initial increase in water content in WMHs.



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Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 614.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 AG058081
NIH RF1 AG056976
University of Minnesota College of Pharmacy

Title: TREM2 modulates amyloid distribution between parenchyma and vasculature in the brain of a mouse model of AD and CAA

Authors: *R. ZHONG, S. MARTIN, A. GRAM, L. LI;
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Abstract: Background: Cerebral amyloid angiopathy (CAA) features cerebral vascular deposition of pathological amyloid-beta protein (Aβ) and plays an important role in the multimorbidity of aging brains including Alzheimer's disease (AD), but it is less understood compared to parenchymal Aβ plaques. Triggering receptor expressed on myeloid cells 2 (TREM2), a key player in innate immune response, is exclusively expressed in microglia in the brain and mediates Aβ clearance and subsequently alleviates neuroinflammation. Loss-of-function mutations in TREM2 increase the risk of AD. Recent studies on TREM2 function using TREM2 knockout (KO) in transgenic (Tg) AD mice have shown that TREM2 modulates Aβ deposition in the brain parenchyma, although the direction of the effect depends on the specific Tg background and the stage of the disease development. However, the impact of TREM2 on CAA had not been investigated. **Methods:** TREM2 KO (TKO) mice were crossed with SwDI mice (C57BL/6-Tg(Thy1-APPSwDutIowa)BWevn/Mmjax), a model of AD and CAA, to generate SwDI/TWT, SwDI/THet, and SwDI/TKO mice. At the age of 16 months, the mice were euthanized for tissue collection. ELISA was utilized for the assessment of Aβ40 and Aβ42 levels in brain tissue homogenates, and (immune)histochemical and immunoblot assays were used throughout the study for characterization and mechanisms. We also assess transcriptomic alterations using single nucleus RNA (snRNA) sequencing. All results were analyzed using GraphPad Prism 9 and/or R. **Results:** TREM2 deficiency markedly increased overall Aβ load in SwDI/TKO mice compared to SwDI/TWT or SwDI/THet in brain cortex, hippocampus, and thalamus. Intriguingly, complete TREM2 deletion led to a dramatic decrease in CAA in vasculature-enriched regions, such as thalamus, along with reduced microglial association with CAA. Consistent with previous reports, Aβ plaque-associated microglia were significantly reduced in SwDI/TKO mice. We also found a significantly robust increase in BACE1 and β-CTF of APP in SwDI/TKO mice. Additional experiments and transcriptomic analysis from snRNA sequencing are underway to explore the molecular mechanisms underlying the distinct impact of TREM2 deficiency on parenchymal Aβ plaques and CAA. **Conclusion:** Deletion of TREM2 increases the overall brain Aβ load via, at least partly, Aβ production whereas decreases CAA,

suggesting the differential role of TREM2 in the regulation of parenchymal versus vascular deposition of Aβ in the brain.

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Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 614.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The impact of chronic hypertension on small vessel disease - from normal appearing white matter to white matter hyperintensities

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Abstract: Cerebral small vessel disease (SVD) is the major vascular cause of dementia and responsible for 20% of all strokes worldwide. As it still remains challenging to visualize the smallest vessels *in vivo* in human brains, SVD diagnosis relies on MRI hallmarks. White matter hyperintensities (WMH) are the foremost common hallmarks of SVD. Despite the impact of SVD on cognition, little is known about the underlying pathology of WMH. This study aimed to investigate the impact of chronic hypertension (HTN), one of the main risk factors for SVD, on vascular pathology, neuroinflammation, and myelin changes in normal appearing white matter (NAWM) and WMH. Therefore, we conducted 7 Tesla high field magnetic resonance imaging (MRI), followed by histopathological stainings on human *post mortem* brains (n = 22) of elderly people with history of chronic HTN and age-matched normotensive individuals. We assessed vascular pathology through Masson's trichrome staining (MTS) and Glucose transporter 1 (GLUT1), which are indicators of collagenosis and vascular endothelial quality/density, respectively. Next, we examined neuroinflammatory markers of astrogliosis, i.e., glial fibrillary acidic protein (GFAP), and microglial activation, i.e., ionized calcium binding adaptor molecule 1 (IBA1). Finally, we assessed myelination with luxol fast blue (LFB). Analysis of LFB solely

allows qualitative assessment of myelin pallor, as it cannot discern densely packed myelin bundles. Therefore, we additionally conducted polarized light imaging (PLI) to accurately assess myelination and orientation of axons based on the birefringent properties of myelin. The resulting multimodal imaging dataset was carefully co-registered and realigned through a custom written MATLAB script to examine MRI-based changes across white matter regions at microscopic resolution. In all individuals, WMH were characterized by increased neuroinflammatory markers, thicker vessel walls, lower vascular density, and myelin loss, compared to surrounding NAWM. Remarkably, individuals with a history of chronic HTN showed a greater neuroinflammatory response in WMH and, to a lesser extent, in areas at risk of developing into WMH, i.e., NAWM, compared to age-matched controls, suggesting that neuroinflammation could play a key role in the progression of WMH in individuals with chronic HTN. Together, these findings provide novel understanding of the impact of HTN on SVD and highlight the benefits of using PLI to characterize white matter.

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Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

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Program #/Poster #: 614.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG055449
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NIA P30 AG072946
NIGMS S10 OD023573

Title: Whole-brain, voxel-wise associations between water exchange rate across the blood-brain barrier and cognitive performance in healthy older adults

Authors: *V. ZACHARIOU[1], C. PAPPAS[1], X. SHAO[2], D. J. WANG[3], B. T. GOLD[1]; [1]Neurosci., Univ. of Kentucky, Lexington, KY; [2]Keck Sch. of Med., USC, Los Angeles, CA; [3]Biomed. Engin., UCLA, Los Angeles, CA

Abstract: The blood-brain barrier (BBB) is crucial for controlling the exchange of compounds between the blood and brain parenchyma and for filtering unwanted toxins and pathogens from entering the brain. Normal aging has been associated with breakdown of the BBB and this disruption has been linked with age-related cognitive disorders such as vascular dementia (VD) and cerebral small vessel disease (cSVD). However, the degree to which age-related BBB

disruptions are associated with cognitive decline in healthy older adults is still under investigation. Here, using voxel-wise linear regressions (at qFDR < 0.05) we evaluate associations between uniform data set (Version 3) based executive function (EF) and episodic memory (MEM) composite scores, and water exchange rate across the BBB (kw) in a cohort of 49 healthy older adults (age range 61-83), while controlling for participant age and gender. BBB kw was quantified using a novel but validated, noninvasive diffusion prepared arterial spin labeling (DP-ASL) MRI method using a Siemens Prisma 3T MRI scanner (64-channel head coil). We found that BBB kw within bilateral regions of the superior frontal gyrus and precuneus was positively associated with EF. Similarly, BBB kw within bilateral regions of the superior frontal gyrus was associated positively with MEM. In contrast to these positive associations between cortical BBB kw and cognition, subcortical BBB kw within the basal ganglia associated negatively with both EF and MEM. These negative associations are in keeping with previous VD and cSVD focused studies showing positive associations between BBB permeability in the basal ganglia, cognitive decline and white-matter hyperintensity (WMH) burden. Therefore, additional analyses were conducted between BBB kw and WMH volumes segmented from 3D fluid-attenuated inversion recovery (FLAIR) images acquired in the same session as the DP-ASL scan, using the ADNI/UCD WMH segmentation toolkit (Version 1.3). These analyses revealed a positive association between basal ganglia kw and periventricular, but not deep WMH volumes. Frontal and parietal lobe kw did not correlate significantly with WMH volume. In sum, the positive associations between cortical BBB kw and cognition are consistent with previous findings from our group showing positive associations between BBB kw and CSF amyloid-β in the same cortical brain regions and may reflect normal BBB clearance functions. Conversely, the basal-ganglia findings may be indicative of BBB disruption and/or leakage, contributing to cognitive decline. Future studies with larger sample sizes should evaluate this possibility further and the capacity of BBB kw as a biomarker of BBB health.

Disclosures: V. Zachariou: None. **C. Pappas:** None. **X. Shao:** None. **D.J. Wang:** None. **B.T. Gold:** None.

Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG064228
NIH R01AG060049
NIH P50AG016573
NIH P01AG052350
AARG-17-532905
CIHR DFD-170763

Title: Enlarged perivascular spaces and cerebrovascular reactivity deficits in older adults

Authors: *A. KAPOOR[1], B. YEW[2], J. JANG[3], S. DUTT[2], Y. LI[1], J. ALITIN[1], A. GAUBERT[1], J. HO[1], A. E. BLANKEN[2], I. SIBLE[2], A. MARSHALL[2], X. SHAO[2], M. MATHER[2], D. J. WANG[4], D. A. NATION[1];
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Abstract: Background: Enlarged perivascular spaces on brain magnetic resonance imaging (MRI) may indicate poor fluid drainage in the brain and have been associated with numerous cerebrovascular and neurodegenerative conditions. Poor fluid drainage and accumulation of waste along perivascular spaces may restrict blood vessel constriction and dilation, and impede the ability of blood vessels to respond to changes in cerebral blood flow. Few prior studies have examined associations between perivascular spaces and dynamic measures of cerebrovascular function. Cerebrovascular reactivity is a marker of cerebrovascular function and represents the ability of cerebral blood vessels to regulate cerebral blood flow (CBF) in response to vasodilatory or vasoconstrictive stimuli. We aimed to examine whether pathological widening of the perivascular space in older adults may be associated with cerebrovascular reactivity. Methods: Thirty-seven independently living older adults (mean age = 66.3 years; SD = 6.8; age range 55-84 years; 29.7% male) free of dementia or clinical stroke were recruited from the community and underwent brain MRI, neuropsychological assessment and blood draw. Visually guided breath control exercises during pseudo-continuous arterial spin labeling MRI were utilized to determine global (whole brain) CVR to hypocapnia (0.1Hz paced breathing; vasoconstriction) and hypercapnia (15s breath holds, vasodilation). Perivascular spaces were qualitatively scored using existing scales. Results: Multiple linear regression analysis revealed a significant negative association between burden of enlarged perivascular spaces and global CVR to hypercapnia (B = -2.0, 95% CI (-3.6, -0.4), p = 0.015), adjusting for age and sex. However, perivascular spaces were not related to CVR to hypocapnia. Discussion: These findings suggest that enlarged perivascular spaces are associated with deficits in CVR, specifically in the vasodilatory response. Accumulation of waste, protein and debris along the perivascular space—which leads to enlargement of the space—may impede the extent to which the blood vessel is able to dilate. Conversely, deficits in cerebrovascular function, indicated by reduced CVR may either contribute to or be indicative of deficits in perivascular fluid homeostasis. Perivascular spaces support the activities of the glymphatic system and dysfunction along these spaces could damage the vasculature and influence CVR. Additional longitudinal studies are warranted to further explore this association and the implications of this association for the development of cerebrovascular and neurodegenerative conditions.

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Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

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Program #/Poster #: 614.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG044404

Title: The role of RhoA/ROCK Signaling Pathway in Oligomeric Aβ and Tau-Induced Inflammatory Response and Subcellular Mechanical Alterations of Cerebral Endothelial Cells

Authors: F. HOSSEN[1], *J. C. LEE[2];
[1]Biomed. Engin., Univ. of Illinios, Chicago, Chicago, IL; [2]Biomed. Engin., Univ. of Illinois, Chicago, Chicago, IL

Abstract: Dysfunction of Cerebral Endothelial Cells (CECs) has been implicated in Alzheimer's disease (AD). Oligomeric amyloid-beta (oAβ) and tau (oTau) have been found playing multiple roles in the AD pathology, yet the mechanism(s) underlying their effects on CECs are not fully understood. In this study, we examined the effects of Aβ and Tau oligomers on primary mouse CECs. Cells were treated with oAβ (1 μM), oTau (0.2 μM) and their combined (oAβ+oTau) followed by cell mechanical and biochemical characterizations. Employing atomic force microscopy (AFM) with cantilever tips biofunctionalized by sialyl-Lewis^x (sLe^x), we found that treatments with oAβ, and oTau for 30 min increased cell stiffness, forces for membrane tether formation and adhesion probability through the p-selectin/sLe^x bonding at the cell surface. Consistent with cell mechanics alterations, these treatments increased actin polymerization, and the expression of p-selectin at the cell surface. In addition, both oAβ and oTau increased inflammation and triggered the RhoA/ROCK pathway, as indicated by significant upregulations of IL1β, TNFα and p-NF-kB p65, and p-RhoA, GTP-RhoA and p-ROCK2, respectively. While these cell mechanical, and biochemical alterations were further enhanced by the combined treatment (oAβ+oTau), they were significantly suppressed by the RhoA/ROCK inhibitor, Fasudil. In sum, our data suggest that oAβ, oTau and their combined treatment triggered inflammatory responses and subcellular mechanical alterations in CECs through the RhoA/ROCK pathway.

Disclosures: F. Hossen: None. **J.C. Lee:** None.

Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 614.07

Topic: C.09.Stroke

Support: NSF 1916894
UTHealth New Faculty Start-up

Title: Leucine zipper-EF-hand containing transmembrane protein 1 is altered in cerebrovascular cells in ischemic stroke

Authors: *X. REN[1], H. HU[2], C. TAN[1], L. MACULLOUGH[1], A. GUSDON[1], R. KITAGAWA[1], A. CHOI[1];
[1]Univ. of Texas Hlth. Sci. Center, Houston, Houston, TX; [2]Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: Background: Ischemic stroke initiates cerebrovascular cell (CEC) injury and blood-brain barrier (BBB) damage resulting in insufficient energy supply and neuronal death. We have shown that maintenance of mitochondrial energy production in CECs is critical for BBB integrity. Leucine zipper-EF-hand containing transmembrane protein 1 (LETM1) is essential for maintenance of mitochondrial structure and membrane potential (ΔΨm). However, the role of LETM1 after stroke is unknown. **Methods:** Human brain sections from acute stroke patients (within 3 days of stroke onset) were compared with age-matched controls (similar postmortem interval). Sections were stained for LETM1 with immunolocalization with the CEC marker (CD31) and visualized by confocal microscopy. Human CECs (hCECs) and murine CECs (mCECs) were cultured and an *in vitro* ischemic stroke model (oxygen-glucose deprivation, OGD) was employed. Mitochondrial oxidative phosphorylation (OxPhos) was evaluated using Seahorse (XFe96) and ΔΨm was evaluated by flow cytometry. An *in vivo* murine stroke model (60 min transient middle cerebral artery occlusion) was employed and co-staining of LETM1/CD31 was performed on mouse brain sections. **Results:** LETM1 is broadly expressed in both CD31 positive and negative cells in control human brain sections. However, LETM1 is almost absent in $CD31^+$ CECs from stroke patients while CD31 negative cells broadly express LETM1. OGD profoundly compromised OxPhos and impaired ΔΨm in hCECs ($p<0.01$, n=6/group) and mCECs ($p<0.01$, n=6/group) at 6 hours post-ischemia. The levels of LETM1 in hCECs ($p<0.01$, n=4/group) and LETM1 in mCECs ($p<0.01$, n=4/group) were significantly decreased at 6 hours post-OGD. LETM1 was also absent in $CD31^+$ CECs from murine stroke brains. **Conclusion:** LETM1 is reduced in CECs from stroke patients. Further, we have shown that LETM1 is reduced in human and murine CECs after ischemia both *in vitro* and *in vivo*. These novel data suggest that LETM1 may play an important role in maintaining BBB integrity and may contribute to stroke outcomes. Future studies will directly manipulate LETM1 levels to determine its functional role in stroke

Disclosures: X. Ren: None. **H. Hu:** None. **C. Tan:** None. **L. MaCullough:** None. **A. Gusdon:** None. **R. Kitagawa:** None. **A. Choi:** None.

Poster

614. The Vasculature and Blood Brain Barrier in Stroke and Cognitive Disease

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 614.08

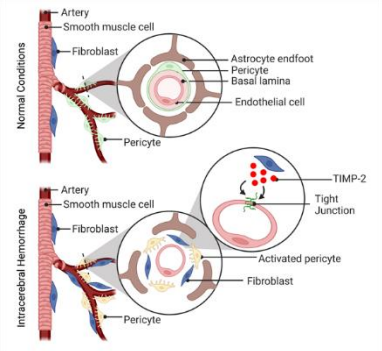
Topic: C.09.Stroke

Support: NIH Grant R01HL146574
NIH Grant RF1AG065345
NIH Grant R21AG073862
NIH Grant R21AG064422

Title: Fibroblasts repair blood-brain barrier damage in intracerebral hemorrhage partially via TIMP2

Authors: L. XU[1,2], ***Y. YAO**[1,2];
[1]Mol. Pharmacol. and Physiol., Univ. of South Florida Morsani Col. of Med., Tampa, FL; [2]Univ. of Georgia, Athens, GA

Abstract: The function of fibroblasts in intracerebral hemorrhage (ICH) remains unknown. By targeting Col1α1---a recently identified fibroblast-specific marker, we generated fibroblast-ablated mutant mice. These mutants showed exacerbated blood-brain barrier (BBB) damage, enlarged injury volume and worse neurological function at the subacute stage, highlighting a beneficial role of Col1α1$^+$ fibroblasts in ICH. Echoed with these findings, fibroblasts significantly decreased endothelial permeability in an *in vitro* ICH model. Next, we demonstrated that fibroblasts promoted BBB integrity in ICH mainly via up-regulating tight junction proteins without affecting transcytosis-associated proteins, indicating a paracellular rather than transcellular mechanism. Subsequent mechanistic study revealed that the BBB-protecting effect of fibroblasts is partially mediated by TIMP2. Furthermore, we found that exogenous TIMP2 attenuated BBB disruption in fibroblast-ablated mice after ICH. Together, these results suggest that Col1α1$^+$ fibroblasts repair BBB damage in ICH via the paracellular pathway in a TIMP2-dependent manner, and that fibroblasts and TIMP2 may be targeted in the treatment of ICH.



Disclosures: L. Xu: None. **Y. Yao:** None.

Poster

615. Alzheimer's Disease Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 615.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant AG054564
NIH grant AG047779
Marie Sklodowska-Curie grant 799638

Title: The proto-oncogene DEK is a modulator of neuronal excitability and tau accumulation in Alzheimer's disease vulnerable neurons.

Authors: *P. RODRIGUEZ RODRIGUEZ[1], L. E. ARROYO-GARCIA[1], L. LI[2], C. TSAGKOGIANNI[1], W. WANG[3], I. SALAS-ALLENDE[3], Z. PLAUTZ[3], A. CEDAZO-MINGUEZ[1], S. C. SINHA[5], O. TROYANSKAYA[6], M. FLAJOLET[4], V. YAO[2], J.-P. ROUSSARIE[7];
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Abstract: Neurons from layer II of the entorhinal cortex (ECII) are the first to accumulate tau protein aggregates and degenerate in Alzheimer's disease (AD. To identify potential drivers of AD pathology in ECII neurons, we used a data-driven functional genomics approach that identified DEK proto-oncogene as a potential driver of tau pathology in ECII neurons. Our results show that downregulation of *Dek* in EC neurons *in vitro* and *in vivo* leads to changes in the inducibility of immediate early genes and alters neuron excitability, triggering the dysregulation of neuronal plasticity genes. Furthermore, these alterations are accompanied by tau accumulation in the soma of ECII neurons *in vivo,* microglia reactivity into a pro-inflammatory and phagocytic profile, and eventually microglia-mediated ECII neuron loss. Together, our results identify *Dek* proto-oncogene as a novel regulator of neuronal plasticity and synaptic transmission which deficiency leads to AD-associated pathological alterations.

Disclosures: P. Rodriguez Rodriguez: None. **L.E. Arroyo-Garcia:** None. **L. Li:** None. **C. Tsagkogianni:** None. **W. Wang:** None. **I. Salas-Allende:** None. **Z. Plautz:** None. **A. Cedazo-Minguez:** None. **S.C. Sinha:** None. **O. Troyanskaya:** None. **M. flajolet:** None. **V. Yao:** None. **J. Roussarie:** None.

Poster

615. Alzheimer's Disease Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 615.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FWO Grant G045420N
SAO-FRA Grant 20180003

Title: Altered information flow from the forebrain in the TgF344-AD rat model of Alzheimer's disease at pre- and early-plaque stages.

Authors: *M. ADHIKARI[1], M. VAN DEN BERG[1], G. KELIRIS[1,2], M. VERHOYE[1]; [1]Univ. of Antwerp, Univ. of Antwerp, Antwerp, Belgium; [2]Fndn. for Res. & Technol. - Hellas, Inst. of Computer Sci., Heraklion, Greece

Abstract: Alzheimer's disease (AD), marked by extracellular accumulation of amyloid-beta (Aβ) plaques, leads to progressive loss of memory and cognitive function. Resting-state (RS) functional connectivity (FC), is emerging as an early biomarker of AD[1]. However, RS-FC measures only correlative relationships between regional neuronal activities. Here, we investigated changes in the causal information flow inferred from RS-fMRI measurements in the TgF344-AD rat model of AD at pre-and early-plaque stages. RS-fMRI data (9.4T Bruker Biospec, 1000 volumes, TR=0.6s) were acquired in anesthetized 4(N=15)- and 6(N=13)-month old TgF344-AD (TG) rats and wild-type (WT) littermates (N=11). Preprocessed voxel-level data were parcellated into 126 grey-matter parcels from the cortex, forebrain, midbrain, hindbrain, hippocampus, olfactory bulb, and cerebellum. Normalized directed transfer entropy (NDTE)[2], an information-theoretic measure of Granger causality, was used to infer directed information flow between region pairs. The predictive cross-validated accuracy of NDTE and FC to classify genotypes at each age was compared. Salient connections in NDTE's predictive accuracy were identified and their median NDTE was compared between genotypes using Wilcoxon rank-sum test. NDTE is calculated using lagged covariances. On average, the autocorrelations of parcels' RS-signals decayed to their first minima in all rats at 9.6s (16 TRs). Therefore, a time window of 9.6 s from the past of all parcels was considered optimal for NDTE calculation. Both NDTE and FC distinguished WT and TG rats better than the chance level at 4-months. At 6-months, only NDTE performed better than chance with 80% accuracy. NDTE accurately classified the two ages only in the TG group, implying changes with age predominantly occur in the TG. Efferent connections from the forebrain, and specifically the globus pallidus, and the bed nucleus of stria terminalis located in the basal forebrain, were salient in NDTE's genotypes classification at both ages. Interestingly, median NDTE for these connections was significantly ($p < 0.05$ FDR) higher in the TG at 4-months but lower at 6-months. Early impact[3] of AD on the basal forebrain, an important modulator of RS networks[4], could explain this altered information flow from it.

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2. Deco, G., Vidaurre, D. & Kringelbach, M. L. *Nature Human Behaviour* 1-15 (2021) doi:10.1038/s41562-020-01003-6.3. Hall, A. M., Moore, R. Y., Lopez, O. L., Kuller, L. & Becker, J. T. **4**, 271-279 (2008).4. Nair, J. *et al. Proc Natl Acad Sci U S A* **115**, 1352-1357 (2018).

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Poster

615. Alzheimer's Disease Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 615.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: In mild cognitive impairment, tau and inflammation are elevated in similar brain regions

Authors: B. PASCUAL, J. APPLETON, Q. FUNK, K. BRADBURY, P. ZANOTTI-FREGONARA, M. FUJITA, ***J. MASDEU**;
Houston Methodist Res. Inst., Houston, TX

Abstract: Background: Neuroinflammation, along with beta amyloid and tau brain deposition, is a potential diagnostic biomarker and therapeutic target in patients with Alzheimer's disease (AD). The role of inflammation at various stages of the AD process needs to be clarified. TSPO PET is useful to image brain inflammation, but some tracers have low affinity for TSPO and "second generation" tracers, with higher affinity, cannot image subjects with a low-binder TSPO rs6971 genotype. We overcame this problem by using ^{11}C-ER176, a high-affinity tracer allowing for imaging of all participants and with a more favorable metabolite profile than other high affinity tracers (Fujita, 2017). Then, we correlated the localization of neuroinflammation tau and amyloid in a subset of the participants.**Methods:** We imaged 15 patients with amnestic MCI (mean age 63±5, 7 women) and 15 healthy controls (HC) (8/15 women, mean age 65 ±7) using ^{11}C-ER176 PET. TSPO affinity was similar (MCI/HC = 2/3 low, 8/7 mixed, 5/5 high). A full factorial analysis was performed on V_T values between groups at the regional level (Hammer's atlas). Ten MCI patients had also amyloid PET (^{18}F-florbetaben or ^{18}F-florbetapir) and tau PET (^{18}F-flortaucipir). Standard uptake value ratios (SUVRs) were calculated for both tracers using the cerebellum as reference. Regional correlations among the three tracers were determined for each patient and an average calculated. All images were corrected for partial volume effect.**Results:** Neuroinflammation in MCI was bilaterally increased in precuneus and lateral temporo- parietal cortex, and right amygdala. There was a correlation between the localization of amyloid and tau in the brain (r=0.61). However, the correlation between neuroinflammation and tau was even higher (r=0.73); neuroinflammation also co-localized with amyloid, but not as strongly (r=0.59). **Conclusion:** ^{11}C-ER176 PET allowed for the identification in MCI of neuroinflammation in regions known to be involved in the AD process. Importantly, all subjects with any TSPO genotype could be studied. As expected, the localization of amyloid and tau in the brain was correlated, but the co-localization of neuroinflammation with tau was even higher than between amyloid and tau. This finding highlights the importance of neuroinflammation as a biomarker of neurodegeneration and as a therapeutic target.

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Poster

615. Alzheimer's Disease Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 615.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brockman Foundation grant
R01 grant (1R01AG067741-01)

Title: Preserving nicotinamide adenine dinucleotide levels protects the blood brain barrier and blocks neurodegeneration and neuropsychiatric impairment in mouse models of early and mid-stage Alzheimer's disease

Authors: *K. CHAUBEY[1,2,3,4], E. VÁZQUEZ-ROSA[1,2,3,4], M.-K. SHIN[1,2,3,4], Y. YU[1,2,3,4], M. DHAR[1,2,3,4], C. CINTRÓN-PÉREZ[1,2,3,4], K. FRANKE[1,2,3,4], X. ZHU[5], B. WILSON[3], Z. BUD[1,2,3,4,6], E. MILLER[1,2,3,4,7], S. BARKER[1,2,3,4,5], Y. KOH[1,2,3,4], S. ROSE[1,2,3,4], H. FUJIOKA[8], X. WANG[5], M. FLANAGAN[9,10], J.-A. WOO[5], D. KANG[5], A. PIEPER[1,2,3,4,5];
[1]Harrington Discovery Institute, Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH; [2]Dept. of Psychiatry Case Western Reserve Univ., Cleveland, OH; [3]Geriatric Res. Educ. and Clin. Ctr. (GRECC), Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [4]Inst. for Transformative Mol. Medicine, Sch. of Medicine, Case Western Reserve Univ., Cleveland, OH; [5]Dept. of Pathology, Sch. of Medicine, Case Western Reserve Univ., Cleveland, OH; [6]Frances Payne Bolton Sch. of Nursing, Case Western Reserve Univ., Cleveland, OH; [7]Dept. of Neuroscience, Case Western Reserve University, Sch. of Med., Cleveland, OH; [8]Cryo-Electron Microscopy Core, Case Western Reserve University, Sch. of Med., Cleveland, OH; [9]Mesulam Ctr. for Cognitive Neurol. and Alzheimer's Disease, Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL; [10]Dept. of Pathology, Northwestern Univ., Chicago, IL

Abstract: There is a profound lack of effective treatments for Alzheimer's disease (AD), which is characterized by both BBB deterioration and neurodegeneration in the brain. Impaired homeostasis of nicotinamide adenine dinucleotide (NAD^+) levels impairs both the integrity of endothelial cells of the blood-brain barrier (BBB) and the survival of neurons in the brain. Here, we demonstrate that brain NAD^+ levels are significantly decreased in both human AD brain and in the brains of male and female 5xFAD mice, a laboratory model of AD. In 5xFAD mice, we tested the hypothesis that restoration of brain NAD^+ levels with the NAD^+-stabilizing agent P7C3-A20 would be therapeutically efficacious. One group of mice received daily P7C3-A20 from 2 to 6 months of age ("early-disease"), and another group of mice received daily P7C3-A20 from 6 to 12 months of age ("mid-disease"). In both groups, treatment with P7C3-A20 preserved the BBB and prevented neurodegeneration, as demonstrated with both immunohistochemistry and electron microscopy, as well as expression levels of tight junction proteins of the BBB. This protection was associated with protection from cognitive decline and acquisition of depression-like and aberrant anxiety-like behavior, as well as reduced neuroinflammation and amyloid plaque accumulation, and restored synaptic transmission. In conclusion, the study showed the efficacy of P7C3-A20 treatment in the early-disease group, before behavioral symptoms are manifest, indicates that NAD^+-based therapy may prevent onset of AD in patients. Efficacy of P7C3-A20 treatment in the mid-disease group, in which treatment is not initiated until after behaviorally symptoms are prominently manifest, indicates that NAD^+-based therapies may prevent AD progression, or even promote disease reversal.

Disclosures: **K. Chaubey:** None. **E. Vázquez-Rosa:** None. **M. Shin:** None. **Y. Yu:** None. **M. Dhar:** None. **C. Cintrón-Pérez:** None. **K. Franke:** None. **X. Zhu:** None. **B. Wilson:** None. **Z. Bud:** None. **E. Miller:** None. **S. Barker:** None. **Y. Koh:** None. **S. Rose:** None. **H. Fujioka:** None. **X. Wang:** None. **M. Flanagan:** None. **J. Woo:** None. **D. Kang:** None. **A. Pieper:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); hold patents related to P7C3 compounds.

Poster

615. Alzheimer's Disease Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 615.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG065240
NIH Grant NS115903
NIH Grant AG076051
NIH Grant AG074346

Title: A CHCHD6-APP axis connects amyloid and mitochondrial pathology in Alzheimer's disease

Authors: *Y. SHANG[1], X. SUN[2], X. CHEN[2], R. XU[2], X. QI[1];
[1]Case Western Reserve Univ., [2]Case Western Reserve Univ., Cleveland, OH

Abstract: The mechanistic relationship between amyloid-beta precursor protein (APP) processing and mitochondrial dysfunction in Alzheimer's disease (AD) has long eluded the field. Here, we report that coiled-coil-helix-coiled-coil-helix domain containing 6 (CHCHD6), a core protein of the mammalian mitochondrial contact site and cristae organizing system, mechanistically connects these AD features through a circular feedback loop that lowers CHCHD6 and raises APP processing. In cellular and animal AD models and human AD brains, the APP intracellular domain fragment inhibits CHCHD6 transcription by binding its promoter. CHCHD6 and APP bind and stabilize one another. Reduced CHCHD6 enhances APP accumulation on mitochondria-associated ER membranes and accelerates APP processing, and induces mitochondrial dysfunction and neuronal cholesterol accumulation, promoting amyloid pathology. Compensation for CHCHD6 loss in an AD mouse model reduces AD-associated neuropathology and cognitive impairment. Thus, CHCHD6 connects APP processing and mitochondrial dysfunction in AD. This provides a potential new therapeutic target for patients.

Disclosures: **Y. Shang:** None. **X. Sun:** None. **X. Chen:** None. **R. Xu:** None. **X. Qi:** None.

Poster

615. Alzheimer's Disease Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 615.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG030142
NCI CCSG P30 CA060553

Title: The role of membrane repair protein annexin A6 in dystrophic neurite formation in Alzheimer's disease

Authors: *K. R. SADLEIR[1], A. KHATRI[1], R. ANDRINGA-SEED[1], A. R. DEMONBREUN[2], E. M. MCNALLY[3], R. VASSAR[1];
[1]Neurol., [2]Pharmacol., [3]Med., Northwestern Univ., Chicago, IL

Abstract: The Alzheimer's disease brain is characterized by amyloid plaques consisting of the β-amyloid peptide, and neurofibrillary tangles containing hyperphosphorylated, aggregated tau. Amyloid plaques form first and likely give rise to tangles, but the mechanistic link between them is unclear. The peri-plaque environment is toxic to neurons, characterized synaptic loss, activated microglia, and vesicle-filled dystrophic neurites, which accumulate aggregation-prone phosphorylated forms of tau. We hypothesize that axonal contact with plaque β-amyloid causes membrane damage, leading to calcium influx, kinase activation, microtubule disruption, trafficking impairment, tau hyperphosphorylation, and dystrophic neurites. Annexin A6 plays a key role in protection against membrane damage in muscle, so we investigated the role of annexin A6 in neuronal repair and dystrophic neurite formation in the 5XFAD amyloid mouse model. Primary neurons from mice expressing genomically encoded annexin A6-GFP were subjected to laser injury to induce membrane damage. After membrane injury, genomic and recombinant annexin A6 localized to the site of damage. In 5XFAD mice, genomic A6-tGFP and endogenous A6 localized to plasma membranes of large neurons and of dystrophic neurites. Overexpression of A6-GFP in neurons in vivo was induced by intracerebroventricular injection of P0 mouse pups which were harvested at 4 months of age for analysis by immunoblot and immunofluorescence. Overexpression of annexin A6-GFP significantly reduced the amount of LAMP1 and ptau-181 positive dystrophic neurites per plaque, while microglia and astrocytes around the plaques remain unchanged. Average dystrophy size was also reduced by A6-GFP expression. These data support our hypothesis that enhanced membrane repair could limit the toxicity of amyloid plaques by reducing dystrophic neurites, lowering pathological tau phosphorylation, which would decrease tau pathology associated with cognitive decline. Further work will explore the ability of annexin A6 to prevent tau spreading, and the use of recombinant A6 as an AD therapeutic.

Disclosures: K.R. Sadleir: None. **A. Khatri:** None. **R. Andringa-Seed:** None. **A.R. Demonbreun:** None. **E.M. McNally:** None. **R. Vassar:** None.

Poster

615. Alzheimer's Disease Mechanisms

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant T32-NS095775
NIH Grant R01-AG068395
Alzheimer's Drug Discovery Foundation Grant GC-201804-2015209
NIH Grant R01-HL119813
Alzheimer's Association Research Grant AARG-16-441560

Title: Identification of novel small-molecule, endogenous ligands of the AD-associated microglial receptor TREM2 that increase its affinity for ApoE and induce cytoprotective immune activation

Authors: *H. B. DEAN[1], R. A. GREER[1], J. A. GREVEN[4], G. N. EASTEP[2], D. S. ELSTON[2], T. J. BRETT[5], Y. SONG[1], E. D. ROBERSON[3];
[1]Dept. of Biomed. Engin., [3]Dept. of Neurol., [2]Univ. of Alabama at Birmingham, Birmingham, AL; [5]Div. of Pulmonary and Critical Care Medicine, Dept. of Intrnl. Med., [4]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) is one of the strongest genetic risk factors for late-onset Alzheimer's disease (AD). While its interactions with known ligands including charged lipids and another AD-associated protein Apolipoprotein E (ApoE) are hotly studied, the full range of endogenous ligands for TREM2 remains unknown. Here we used in silico virtual screening of small molecule metabolites found in the human body followed by molecular dynamics-molecular mechanics Poisson-Boltzmann surface area (MD/MM-PBSA) binding free energy estimation to identify likely endogenous ligands of TREM2. Top hits from the virtual screen were then confirmed to bind TREM2 using biolayer interferometry (BLI). BLI was also used to confirm the predicted binding site(s) on TREM2 by comparing binding of the hits to TREM2 constructs with mutations in amino acids predicted in simulations to be key residues for ligand interactions. Hits found to bind TREM2 were then examined for the ability to induce TREM2-dependent intracellular signaling, phagocytosis, and protection against LPS-induced cytotoxicity in cultured primary mouse macrophages and microglia. We then tested whether small molecule hits protected neurons against amyloid-β oligomer-induced toxicity in primary mouse hippocampal neuron-microglia co-culture. Intracellular signaling, phagocytosis, and cytoprotection by these hits were also examined in primary rat microglia and neuron-microglia co-culture to see whether hits had similar efficacy to known small-molecule ligands of TREM2. Using BLI, we also found some hits that bound TREM2 near the previously identified binding site of ApoE were also shown to enhance ApoE-TREM2 binding, even in the presence of the AD-associated R47H variant. Overall, our studies identify potential endogenous ligands of TREM2 that activate many of the known immune functions previously seen to be activated by known lipid ligands of TREM2 and that may be useful for developing future therapeutics targeting TREM2 in AD. We also show these ligands do not just independently activate TREM2 but also stimulate its ability to bind the protein ApoE, suggesting a potential mechanism by

which small-molecule and protein ligands of TREM2 interact to enhance signaling in AD pathology.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21 AG067008-01
R01 MH099073
2021M3E5D2A01023887
NRF-2022R1A2C2009265

Title: Alterations in behavioral flexibility and hippocampal sharp-wave ripples during naturalistic risky decision-making in a mouse model of Alzheimer's disease

Authors: *E. KIM[1,3], B. P. SCHUESSLER[1], S. PARK[4], J. CHO[4], J. J. KIM[1,2];
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Abstract: While much research on Alzheimer's disease (AD) has focused on neuropathological markers and consequent loss of memory functions, there is limited information as to how AD adversely affects brain cell activities involved in other cognitive functions, such as risky decision-making. Previously, we reported that 5XFAD mouse model of AD, expressing five mutations in human APP and PS1 (4-8 months old male and female mice), showed impairment in discerning safety-danger boundaries and making optimal foraging decisions when tested in ecologically-relevant 'approach food-avoid predator' paradigms (Schuessler et al., 2021). Given the critical role of hippocampal sharp-wave ripples (SWRs) and prefrontal cortical activity in decision-making and "contextual emotional experience" (Schmidt & Redish, 2021; Girardeau et al., 2017), we investigated whether and how SWRs and SWRs-associated neuronal interactions in the prefrontal-hippocampal circuit are altered by amyloid pathology in the 5XFAD mice. To do so, male and female 5XFAD and wild-type (WT) mice, implanted with tetrode arrays in the medial prefrontal cortex (mPFC) and CA1 area of the dorsal hippocampus (dCA1) ipsilaterally, underwent successive sessions of nest habituation, foraging preference baseline, and predator testing in a figure-8 maze where two different pellets (grain-based vs. chocolate-flavored) were

placed in the two arms. Tetrodes were gradually advanced towards their target structures, and neural activities were recorded during the predator testing session, which consisted of pre-predator (8-11 trials), predator (5-8 trials), and post-predator (10 min in nest) stages. During the predator trials, every time the animal approached the preferred pellet, the predator surged forward. In response to the predator, the WT mice switched their foraging strategy from the preferred to non-preferred pellets. In contrast, the 5XFAD mice continued to choose their preferred pellets, indicating an inability to adjust their foraging behavior as a function of danger. During the post-predator trials, 5XFAD mice demonstrated decreased frequency and shortened duration of dCA1 SWRs events compared to the WT mice. However, comparable proportions of mPFC units exhibited SWRs-associated activity in the two groups, indicating that surviving hippocampal SWRs can modulate mPFC activity in the 5XFAD mice. These results of impaired behavioral flexibility and reduced hippocampal SWRs during risky decision-making in AD mice suggest that restoring hippocampal SWRs may be a novel therapeutic strategy for AD.

Disclosures: E. Kim: None. **B.P. Schuessler:** None. **S. Park:** None. **J. Cho:** None. **J.J. Kim:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21 AG067008-01 (E.J.K.)
R01 MH099073 (J.J.K.)

Title: 5XFAD transgenic mouse model of Alzheimer's disease: a longitudinal analysis of naturalistic foraging behavior

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Abstract: Studies utilizing animal models of Alzheimer's disease have primarily focused on the disease's impact on higher cognitive functions, particularly learning and memory. Less studied in animal models is the impact of the disease on other behavioral processes, such as motivation (Boyle et al., 2003), eating pattern (Tsang et al., 2010) and circadian rhythm (Coogan et al., 2013). The present study sought to comprehensively investigate these understudied processes over an extended period using the Five Familial AD (5XFAD) mouse model of Alzheimer's disease. To do so, male 5XFAD transgenic mice (3-4 months old) were housed under closed economy conditions, wherein animals were required to obtain their daily food solely via lever pressing (Schuessler et al., 2020). To assess motivation levels and circadian appetitive (foraging) behavior, a fixed ratio 50-continuous reinforcement (FR50-CRF) chained schedule of

reinforcement was used. Under this schedule, animals were required to initially press 49 times without reinforcement. On the 50th press they were rewarded with a food pellet and entered into the CRF phase, where each press was reinforced with one pellet. This schedule imposed naturalistic food procurement/consumption costs. Additionally, the lever schedule reset after 1 minute of no pressing. This meant animals needed to exert sustained effort to obtain food. Locomotor and foraging behavior in closed economy chambers were measured automatically and near continuously (23-47 hours per day/session) for several weeks. Overall, there were minimal differences between 5XFAD hemizygous mice (n=8) and their wild type littermates (n=8); there were no significant differences between groups in the total amount of food pellets obtained, failed transitions into CRF and distance travelled (locomotor activity) per day. However, 5XFAD mice engaged in significantly more eating bouts per day ("meals") relative to their wild type littermates, which also resulted in significantly more lever presses per day. Analysis of circadian rhythm under reverse 12-hour light/dark conditions shows that while both genotypes displayed comparable circadian behavior, 5XFAD mice foraged more during the last three hours of the dark cycle (ZT 22-24). This study suggests that at 3-4 months of age, male 5XFAD mice do not show general deficits in food motivation or locomotor activity, but rather subtle changes in daily foraging pattern.

Disclosures: B.P. Schuessler: None. **L. Bellmont-Olson:** None. **J.J. Kim:** None. **E. Kim:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS085171
NIH Grant AG065290

Title: Genetic susceptibility to disease progression in a transgenic mouse model of Alzheimer's disease

Authors: *P.-Y. CHUANG, C.-H. FU, J. CHIN;
Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) and tau accumulation, dysregulated neuronal activity, abnormal oscillatory rhythms, and cognitive deficits. However, susceptibility to disease progression differs between individuals with AD. Some patients with AD neuropathology are cognitively normal. Using transgenic mice expressing human amyloid precursor protein (hAPP), a model used to study the role of APP/Aβ in AD, our lab previously discovered that some APP mice have normal memory, despite having similar levels of APP/Aβ as their cognitively impaired siblings. However, the mechanisms underlying such differential

susceptibility remain largely unknown. Previous research discovered that AD patients have increased incidence of seizure activity, the presence of which predicts faster memory decline. Our lab demonstrated that the severity of memory deficits in APP mice corresponds with frequency of spontaneous seizures and expression of the transcription factor ΔFosB. APP mice with frequent seizures and high expression of ΔFosB show worse cognition. Therefore, to investigate the underlying mechanisms of susceptibility in AD, our lab used the expression level of ΔFosB to group APP mice into those with normal level of ΔFosB ($APP_{\Delta FosB\text{-}lo}$) or those with high ΔFosB ($APP_{\Delta FosB\text{-}hi}$) and compared them to nontransgenic control (NTG) mice. RNA-sequencing analysis revealed a large portion of genes that are distinctly differentially expressed in $APP_{\Delta FosB\text{-}hi}$ mice, some of which we predict may represent susceptibility genes. Using an analytic tool called MAGIC (Mining Algorithm for GenetIc Controllers), which predicts transcription factors (TFs) and cofactors responsible for regulating a particular set of genes, we also discovered several TFs that are not only predicted to be the regulators for the differentially expressed genes (DEGs) between $APP_{\Delta FosB\text{-}hi}$ vs $APP_{\Delta FosB\text{-}lo}$ mice but are themselves also upregulated in $APP_{\Delta FosB\text{-}hi}$ mice such as Fosl2, Bach1, and others. We performed gene ontology network analysis on the DEGs predicted to be the target genes of Fosl2 and Bach1 and found them to be mostly related to synaptic transmission, neuron death and response to oxygen level, which are relevant to AD disease progression. A number of these target genes, such as Sema3a, Penk, and Agrn were increased in $APP_{\Delta FosB\text{-}hi}$ mice and have been previously implicated in AD, and we also identified a number of novel genes that are of interest to investigate further. These genes may be potential susceptibility factors for symptom progression in APP mice; understanding their roles could reveal novel targets for the development of effective treatments for AD.

Disclosures: **P. Chuang:** None. **C. Fu:** None. **J. Chin:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1-AG-041374 NIA

Title: Effects of estradiol on memory in the APP^{NLGF} knock-in mouse model of Alzheimer's disease

Authors: *A. F. DELARGE[1,2], A. CHUDY[2,3], A. YOUSHA[2,3], J. M. DANIEL[1,2,3];
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Abstract: Previous work from our lab has shown the capacity of estradiol (E2), the main ovarian estrogen, to enhance cognitive and brain aging in rodent models. Thus, the purpose of the current

study was to determine whether these memory enhancing effects also extend to a rodent model Alzheimer's disease (AD). In order to do this, a transgenic mouse strain containing an amyloid precursor protein (APP) knock-in comprised of 3 mutations (NL-Swedish, G-Artic, F-Beyreuther/Iberian, APP^{NLGF}) was utilized. APP^{NLGF} mice spontaneously develop amyloid beta plaques in the brain starting at 2 months of age, followed by cognitive deficits by 8 months. In the current experiment, APP^{NLGF} female mice and wild type (WT) controls were ovariectomized and implanted with a subcutaneous capsule at 70 days of age. Capsules contained either vehicle (cholesterol) or 2.5% E2 diluted in vehicle. Mice were designated to 1 of 4 experimental groups: WT+vehicle, WT+E2, APP^{NLGF}+vehicle, APP^{NLGF} +E2. Mice underwent acquisition training on an eight-arm radial maze for a period of 24 days. Following training, delay dependent effects on spatial memory were tested by inserting delays of 2, 4 and 6 hours, between the 4^{th} and 5^{th} arm choices. Mice were tested on delay trials at 4 months and again at 6 months of age. Performance was assessed by number of errors (reentry into arm previously entered) made within the first 8 arm choices (errors of 8). Statistical analysis of results from the 4-month delay trial did not reveal any significant differences in memory between the 4 experimental groups. However, there was a trend towards a main effect of hormone status ($p=0.063$). By the 6-month delay trial, data revealed a main effect of hormone status ($p<0.01$). Specifically, ovariectomized mice implanted with E2 capsules made significantly fewer errors within the first 8 arm choices than those implanted with vehicle capsules, regardless of strain. From the current results, we can conclude that the memory enhancing effects of E2 do extend to the APP^{NLGF} knock-in mouse model of AD at early time points and before memory deficits appear in APP^{NLGF} mice. Mice will be tested at additional time points (9 and 12 months of age) to determine if the memory enhancing effects of E2 will persist as amyloid plaque load in the brain of APP^{NLGF} mice increases over time.

Disclosures: A.F. DeLarge: None. **A. Chudy:** None. **A. Yousha:** None. **J.M. Daniel:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant MOP-84480
APRI- ASANT
SynAD postdoctoral fellowships grant

Title: The disease-modifying potential of native-PLGA nanoparticles in the treatment of Alzheimer's disease pathology

Authors: *K. GOVINDARAJAN[1], Q. WU[1], S. WANG[5], A. DAHAL[2], X. LI[1], D. GALLEGUILLOS[3], S. SIPIONE[3], G. THINAKARAN[5], S. KAR[4];
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(Neurology) & Ctr. for Prions and Protein Folding Dis. & NMHI, Univ. of Alberta, Edmonton, AB, Canada; [5]Dept. of Mol. Med. and Byrd Alzheimer's Inst., Univ. of South Florida, Tampa, FL

Abstract: At present, there is no effective treatment for Alzheimer's disease (AD), the most prevalent cause of dementia affecting the elderly. Evidence suggests that enhanced levels and aggregation of beta-amyloid (Aβ) peptide contributes to neurotoxicity and progression of AD. Thus, preventing Aβ aggregation/toxicity may delay the onset/progression of AD. Recently, acidic poly (D, L-lactide-co-glycolide) (PLGA) nanoparticles, a class of FDA-approved biodegradable polymers, have been studied in delivering drugs/agents to the target areas in various diseases. The beneficial effects were attributed to conjugated drugs rather than PLGA nanoparticles. We recently reported that PLGA nanoparticles without any drug (native-PLGA) could suppress Aβ aggregation/toxicity and protect against Aβ inflicted toxicity in primary neurons. Thus, we evaluated the therapeutic potential of native-PLGA in the 5xFAD-Tg mouse model of AD. In this study, eight-month-old 5xFAD-Tg and WT mice were administered native-PLGA or cerebrospinal fluid (CSF) intracerebrally for 28 days using mini-osmotic pumps. After the treatment, animals (CSF-WT; PLGA-WT; CSF-5xFAD-Tg; PLGA-5xFAD-Tg mice) were subjected to cognitive tests, and their brains were processed for various anatomical, biochemical, and molecular analyses. Our results revealed that native-PLGA treatment markedly attenuated cognitive/behavioral impairments (spatial and recognition memory) and various AD-related pathologies in 5xFAD-Tg mice, including increased Aβ levels/deposits, oxidative stress, and plaque-associated microglia possibly by decreasing the levels/expressions of amyloid precursor protein (APP) and its cleaved products, i.e., C-terminal fragment-α (CTFα) and CTFβ in the affected cortical regions but not in the unaffected cerebellar regions. Our RNA-seq data from the affected regions revealed that >2907 genes were altered differentially in native-PLGA vs. CSF-treated 5xFAD animals; based on Gene Set Enrichment Analysis, we observed that some of these alterations are associated with the microglia-neuronal response. These findings provide unequivocal evidence that native-PLGA, by targeting different facets of the Aβ axis, offers unique therapeutic potential in the treating of AD amyloid pathogenesis.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG054551
Martin L. and Sylvia Seevak Hoffman Fellowship

Title: Amyloid-beta's disruption of hippocampal neuronal circuit function depends upon behavioral state

Authors: *H. LI, H. ZHOU, N. GOWRAVARAM, M. QUAN, N. KAUSAR, S. N. GOMPERTS;
Massachusetts Gen. Hosp., Boston, MA

Abstract: The Alzheimer's disease-associated peptide amyloid-beta (Aβ) drives neuronal hyperactivity under anesthesia, but clinical trials of anticonvulsants or neural system suppressors have, so far, failed to improve symptoms in AD. Using simultaneous hippocampal calcium imaging and electrophysiology in freely-moving mice expressing human Aβ, we show that Aβ aggregates perturbed neural systems in a state-dependent fashion, driving neuronal hyperactivity in exploratory behavior and slow wave sleep (SWS), yet suppressing activity in quiet wakefulness and REM sleep. Aβ impaired hippocampal theta-gamma phase-amplitude coupling (PAC) in exploratory behavior and REM sleep; in SWS, Aβ reduced cortical slow oscillation (SO) power and coordination of hippocampal sharp wave-ripples with the SO and thalamocortical spindles. Physostigmine only partially rescued Aβ-associated aberrant calcium activity and theta-gamma PAC. Together, these findings show that Aβ's effects on hippocampal circuit function are profoundly state dependent and suggest a reformulation of therapeutic strategies aimed at Aβ induced hyperexcitability.

Disclosures: H. Li: None. **H. Zhou:** None. **N. Gowravaram:** None. **M. Quan:** None. **N. Kausar:** None. **S.N. Gomperts:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Alzheimer's disease in 3D: characterization of plaques and neuroinflammation within AD mouse models using innovative tissue clearing and imaging techniques

Authors: *M. L. JACOBSON[1], K. AMES[2], A. NAMBIAR[2], L. M. VANDENBOSCH[2], L. A. SCHUBERT[2], T. N. MARTINEZ[1], M. M. MACBRIDE[1], A. C. HALL[2];
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Abstract: Transgenic mouse models are crucial to understanding mechanisms of neurodegenerative disorders and evaluating therapies. Alzheimer's disease (AD) is a multifaceted progressive neurodegenerative disease with many underlying mechanisms. The amyloid cascade hypothesis, where mutations in the amyloid precursor protein (APP) lead to amyloid accumulation (plaques) continues to dominate the research field. Thus, identifying detailed spatial distribution and density of β-amyloid plaques and pathological markers of neuroinflammation is critical to further our understanding of AD.

We aimed to demonstrate proof of concept ex-vivo 3D imaging in mouse models used to interrogate AD by characterizing 3D distribution and densities of β-amyloid plaques and other pathological markers in brains of male and female ARTE10 (APP-PS1) and APPSWE (Tg2576) across different ages. Both models express high concentrations of the β-amyloid precursor protein, result in amyloid plaque depositions, glial activation, and cognitive impairment. ARTE10 animals were assessed at 10 wks, 6 mths, and 10 mths, while APPSWE animals were assessed at 10 wks, 10 mths, and 13 mths. Mice were transcardially perfused with 4% PFA and brain samples dissected. Intact fixed samples were post-fixed with SHIELD, delipidated, stained with syto 16, IBA-1 or GFAP & β-amyloid antibodies, index-matched and imaged using a SmartSPIM light sheet microscope. Whole brain images were acquired with a 3.6x objective, registered to the Allen Brain Atlas and regional cell counts/plaque densities quantified using SmartAnalytics (LifeCanvas Technologies).
3D images and analyses revealed plaques throughout the brains of APPSWE (>10 mths) and ARTE10 mice (>6 mths) compared to controls, with increased densities within cortical areas, hippocampus, and olfactory bulbs. Reactive glia, including microglia (IBA-1+) and astrocytes (GFAP+), clustered around and infiltrated amyloid plaques in both ARTE10 and APPSWE mice. ARTE10 mice developed plaques earlier than APPSWE mice and had more plaques during development. There were no apparent sex differences in ARTE10 mice, but female APPSWE mice had increased plaque deposition relative to males.
Using novel imaging technology, this study produced more robust, unbiased, and high-resolution data with improved characterization of the mouse models than would be possible with traditional 2D immunohistochemistry. By increasing our understanding of disease progression across different models, ages, and sexes, these models and imaging techniques have the potential to improve the evaluation of disease progression and of novel therapeutics.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.08

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: U54 AG054345

Title: Assessing the role of ABCA7*A1527G risk in a novel mouse model late-onset Alzheimer's disease

Authors: *P. R. TERRITO[1], A. OBLAK[1], E. CHUMIN[1], S. PERSOHN[1], A. BEDWELL[1], K. ELDRIDGE[1], R. SPEEDY[1], K. P. KOTREDES[2], R. PANDEY[3], S. J. SUKOFF RIZZO[4], G. W. CARTER[3], B. T. LAMB[5], M. SASNER[3], G. R. HOWELL[3];

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Abstract: Objectives: MODEL-AD is developing more than 40 novel mouse models and combination that better phenocopy human LOAD for preclinical testing. In this study, we aim to determine the mechanisms by which variations in the ABCA7 gene increases LOAD risk.
Methods: MODEL-AD identified the A1527G variant in ABCA7 (ABCA7*A1527G) as a LOAD risk. CRISPR/CAS9 introduced the Abca7*A1527G variant on to B6.APOE4.Trem2*R47H mice (LOAD1), where transcriptional brain profiling from 12 mos mice was used to evaluate molecular effects. This allele was incorporated into LOAD1.hAβ mice (LOAD2) to evaluate the contribution of Abca7.A1527G to LOAD risk. Both sexes of LOAD2.Abca7*A1527G and LOAD2 control mice are being characterized from 4-24 mos via the MODEL-AD phenotyping pipeline (in vivo imaging of brain perfusion and glycolytic metabolism, multi-omics, fluid biomarkers, behavior and neuropathology).
Results: The Abca7*A1527G variant on LOAD1 background showed alignment to human LOAD of brain transcriptomes at 12 mos, and was prioritized for deep phenotyping on the LOAD2 platform. LOAD2 mice showed an age and regional elevation in brain perfusion, without regional increases in glycolysis, between 4-12 mos. Over this same interval, LOAD2.Abca7*A1527G mice showed a significant age and region dependent increase in brain glycolysis with a concomitant decrease in regional perfusion only in female mice yielding an neurovascular uncoupled phenotype compared to LOAD2. Consensus clustering of regional covariance matrices revealed an increase in cluster number and organization in LOAD2.Abca7*A1527G over LOAD2 for both sexes at 4 mos. By 12 mos, cluster number and complexity were reduced by in LOAD2.Abca7*A1527G mice relative to LOAD2. Addition of the Abca7 allele on the LOAD2 background resulted in reduced cluster numbers between 4-12 mos for both sexes, while LOAD2 over this same interval for both sexes was largely unchanged. Plasma Aβ40 and Aβ42 showed an age dependent, but genotype independent, increases. Consistent with the neurovascular uncoupling, cytokines IL6, IL10, and TNFα were elevated in plasma with LOAD2.Abca7*A1527G, but were not age dependent. Interestingly, brain levels of IL4, IL12, TNFα, and CXCL1 were decreased, while IL2 and IL10 were increasing in LOAD2.Abca7*A1527G mice relative to LOAD2 controls. Additional time points (4-24 months) and phenotyping are still in progress.
Conclusions: These data reveal the combination of Abca7*A1527G on a LOAD2 background showed an age-dependent LOAD-relevant neurovascular uncoupling and cytokine phenotype consistent with vascular deficits, making these mice a useful tool for mechanistic and therapeutic studies.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ohio Lions Eye Research Foundation Graduate Student Fellowship
Start-up Funds to C.M.D.C

Title: Investigating the integrity of cholinergic brainstem nuclei in a mouse model of Alzheimer's disease

Authors: *G. FRAME, M. A. SMITH, C. M. DENGLER-CRISH;
Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH

Abstract: Cholinergic dysfunction is a well-documented occurrence in Alzheimer's disease (AD) progression, and loss of cholinergic neurons in the basal forebrain is thought to contribute to the memory deficits associated with AD. However, current knowledge on these cholinergic changes is limited to the basal forebrain nuclei—thus overlooking the potential impact of AD pathogenesis on other major cholinergic cell groups outside the telencephalon. This study sought to characterize how AD pathogenesis impacts the integrity of three major brainstem cholinergic nuclei in mice— the parabigeminal nucleus (PBGN), the pedunculopontine nucleus (PPN), and the laterodorsal tegmental nucleus (LDT). All three cholinergic nuclei are involved in sensory processing across several modalities including vision, audition, and somatosensation and also play roles in arousal, movement, and sleep. As deficits in sensory processing are becoming increasingly recognized in AD, the goal of this project was to link early sensory dysfunction observed in 3xtg AD mice with changes in these cholinergic brainstem nuclei. We used a combination of neurohistological techniques to identify cholinergic cells in the PBGN, PPN, and LDT of 3xtg mice and determined whether of AD-related neuropathology colocalized with cholinergic cells. Using retrograde tract tracing, we also assessed the integrity of projections between these brainstem cholinergic nuclei and the superior colliculus—a structure involved in multi-sensory integration that would likely be impacted by loss of cholinergic input. Overall, data from this project will provide important information on the impact of AD pathogenesis on non-cognitive brain structures and functions—which may be among the earliest deficits reported in dementia.

Disclosures: G. Frame: None. **M.A. Smith:** None. **C.M. Dengler-Crish:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

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Program #/Poster #: 616.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NS085171
NIH NS086965
NIH F31AG063462

Title: Persistent ΔFosB activity is required to restrict seizure activity and maintain neuroprotection in the dentate gyrus of APP mice

Authors: *G. STEPHENS[1], Y. ZHENG[1], J. PARK[1], M. SILVA-PÉREZ[1], A. L. EAGLE[2], J. YOU[1], C.-H. FU[1], C. ST-ROMAIN[1], S. CHOI[1], C. SUGIMOTO[2], S. BUFFINGTON[1], M. COSTA-MATTIOLI[1], Y. LIU[3], A. ROBISON[2], J. CHIN[1];
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Abstract: Recurrent seizures contribute to cognitive deficits in many neurological disorders, including epilepsy and Alzheimer's disease (AD). We reported that recurrent seizures can promote cognitive impairment in part via hippocampal accumulation of ΔFosB, a transcription factor that can epigenetically regulate various target genes in the dentate gyrus (DG) of mice with recurrent seizures, including those with pharmacologically induced epilepsy or transgenic mice used to study AD. We have previously shown that viral blockade of DG ΔFosB activity for 1 month can ameliorate spatial memory deficits in human amyloid precursor protein transgenic mice (J20 APP mice), a mouse model used to study AD, without impacting the spontaneous seizure activity they exhibit. However, we recently reported that hippocampal ΔFosB also binds to many genes involved in key pathways that could regulate neuronal excitability and neuroprotection in APP mice and in Pilocarpine-injected mice with recurrent seizures. Our findings suggested that ΔFosB may play long-acting roles in seizure suppression and neuroprotection in conditions with recurrent seizure activity. We therefore predicted that DG blockade of ΔFosB activity for extended periods of time would exacerbate seizure activity. To test this hypothesis, we used viral strategies to block ΔFosB activity in the DG of APP mice for 1 month or 2-4 months to determine if persistent ΔFosB activity is required to restrict seizure activity and maintain neuroprotection. Unlike APP mice with 1 month blockade of ΔFosB, APP mice with 2-4 month blockade of ΔFosB exhibited increased mortality, spike/seizure activity, memory deficits, DG hyperexcitability, and DG atrophy. In vitro studies confirmed that overexpression of ΔFosB in primary hippocampal neurons conferred neuroprotection after NMDA challenge. Our results indicate that persistent ΔFosB regulation of hippocampal target genes over extended periods of time restricts seizures and maintains neuroprotection in APP mice.

Disclosures: G. Stephens: None. **Y. Zheng:** None. **J. Park:** None. **M. Silva-Pérez:** None. **A.L. Eagle:** None. **J. You:** None. **C. Fu:** None. **C. St-Romain:** None. **S. Choi:** None. **C. Sugimoto:** None. **S. Buffington:** None. **M. Costa-Mattioli:** None. **Y. Liu:** None. **A. Robison:** None. **J. Chin:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

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Program #/Poster #: 616.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Sinai Medical Staff Foundation
the Robert E Nederlander Sr. Program for Alzheimer's Research
the A. Alfred Taubman Medical Research Institute
the NeuroNetwork for Emerging Therapies at Michigan Medicine

Title: Tau hyperphosphorylation through increased amyloid precursor protein in exosomes in a cell culture model of the metabolic syndrome

Authors: *B. KIM[1], E. L. FELDMAN[2];
[1]Univ. of Michigan, [2]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: The US and global prevalence of metabolic syndrome (MetS) has reached epidemic proportions. Among US adults aged 18 years or older, MetS prevalence has risen by over 35% in the last 20 years. MetS, including obesity and diabetes, is a risk factor for developing Alzheimer's disease (AD). An increase in free fatty acids (FFAs), especially saturated FFAs (sFFAs) such as palmitate, is a main characteristic of MetS, and clinical studies demonstrate that higher sFFA levels in plasma inversely correlate with cognitive function and are a significant risk factor for developing AD. Exosomes are a subset of extracellular vesicles that allow intercellular communication and can spread pathological proteins involved in AD, Parkinson's disease, and prion diseases. Amyloid precursor protein (APP), β- & γ-secretases, and tau are found in exosomes derived from AD neurons and microglia. Exosomal proteins accumulate in plaques in AD brains, and inhibition of exosome secretion reduces plaque load in 5XFAD mice. Similarly, extracellular vesicle cargo composition is altered and vesicle production is higher in plasma from humans with MetS, and can elicit inflammation through inter-organ communication between target tissues. These findings indicate that exosomes serve as a point of convergence between MetS and AD. In this study, we used a cell culture model of MetS to examine the possibility that exosomes spread AD pathology among cortical neurons. Cortical neurons developed insulin resistance (IR), a key feature of MetS, after treatment with the sFFA palmitate, as evidenced by decreased Akt phosphorylation and increased JNK and IRS-1 phosphorylation. Palmitate also increased APP phosphorylation at threonine 668, which favors amyloidogenic processing and Aβ production. Palmitate and the non-sFAA oleate both increased secretion of exosomes in culture supernatant (pal-exo and ole-exo, respectively); however, we observed increased levels of total APP and the C-terminal fragment (CTF) of APP only in in pal-exo. Treatment of naïve recipient cortical neurons with pal-exo, but not ole-exo, similarly resulted in a robust increase in tau phosphorylation. Overall, our results suggest that increased levels of APP in exosomes following sFFA treatment represents a possible explanation for the increased risk of AD in MetS, and thereby offers insights into potential mechanisms and new therapeutic targets linking MetS with AD. The authors would like to thank the Sinai Medical Staff Foundation, the Robert E. Nederlander Sr. Program for Alzheimer's Research, the A. Alfred Taubman Medical Research Institute, and the NeuroNetwork for Emerging Therapies at Michigan Medicine for funding.

Disclosures: **B. Kim:** None. **E.L. Feldman:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Society of Canada

Title: Simulation of sporadic late-onset Alzheimer's disease in humanized amyloid-β & apolipoprotein E4 hybrid knock-in mice on a high-sucrose diet

Authors: *A. JAGAIT, L. K. BEKAR;
Anatomy, Physiol. & Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Alzheimer's disease (AD) is a debilitating condition typified by cognitive decline and frequent lapses in one's short-term memory. Genetic analyses have identified a common risk factor amongst over 50% of affected patients: the *Apolipoprotein E4 (APOE4)* allele. Recent studies have also delved into detecting dietary risks, such as sugar consumption, and outlined plausible relationships between metabolic comorbidities and AD. Given the pervasiveness of sugar consumption in Western society and prevalence of *APOE4*, our research investigates the possibility of a gene-environment interaction between these two factors in the genesis of AD. Because wildtype mice do not develop amyloid plaques/aggregates like humans, we employ mice with a humanized amyloid peptide sequence (3 amino acids mutated that enable aggregation) with and without additional *APOE4* expression to explore the above-mentioned gene-environment interaction. Humanized amyloid-β (hAβ), and hybrid Aβ/*APOE4* mice are sustained on 20% sucrose water or control water for five months. Both male and female mice are used to elucidate sex differences. Cognitive effects are assessed via the Barnes maze and modified Y-maze behavioral tests. After six months on the water regimen, animals are sacrificed and half brains are harvested for measurement of cortical acetylcholinesterase and nitric oxide biochemically, while the other half brain is post-fixed in PFA for later immunohistochemical analyses of Aβ aggregation and hyperphosphorylated tau. In this study, the effects of high sucrose water on cognitive behavior and AD-related biochemistry is compared in hAβ and hybrid mice with the *APOE4* allele using a 2x2 design. We hypothesize that both hAβ and hybrid mice will show pronounced displays of AD phenotypes when sustained on high sugar regimens. We also hypothesize that *APOE4* expression will exacerbate the phenotype. This is because these mice express a "humanized" variant of the Aβ gene that allows Aβ aggregation into soluble plaques, mimicking the human condition and improving face validity of AD research. AD-associated phenotypes such as this are further aggravated by the presence of a gene-environment interaction in the hybrid mice through an interplay between the *APOE4* allele and a high sugar diet. In isolation, both factors have been shown to magnify Aβ accumulation through associations with impaired trafficking and insulin signalling. By understanding how these

predisposing factors work in concert, risks can be explored in vulnerable populations, inciting prophylactic inquiry to mitigate the incidence of AD.

Disclosures: **A. Jagait:** None. **L.K. Bekar:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ALF Region Skåne
Swedish brain foundation
Swedish Research council

Title: Prion-like intracellular Aβ and the pathogenesis of Alzheimer's disease

Authors: *T. T. ROOS[1], M. GARCIA[2], G. K. GOURAS[2];
[2]Lund Univ., [1]Lund Univ., Lund, Sweden

Abstract: The neuropathological hallmarks of AD are extracellular plaques primarily made of the peptide Aβ and intracellular neurofibrillary tangles primarily made of the protein tau. Neuropathological, biomarker and genetic evidence suggest that Aβ plays a key role in initiating AD. This position has been increasingly challenged as there is only a weak correlation between plaques and dementia, and several trials of disease-modifying treatments targeting Aβ have successfully reduced plaques but failed in their primary end-point, to stave off dementia. The evidence indicating an important causal role of Aβ in AD needs to be squared with the seeming irrelevance of Aβ plaques.
We have studied the role of intracellular Aβ in the prion-like spread of Aβ. According to the prion-like hypothesis, Aβ can misfold into a prion-like confirmation and spread its pathological confirmation via templated seeding. Many studies show that Aβ plaque formation is accelerated in a prion-like manner by injection of AD brain homogenate into the hippocampus of an AD mouse model. We used the same paradigm in 5xFAD mice (n = 13) but focused on intracellular effects. Hippocampal injection of AD brain homogenate increases intracellular Aβ in the synaptically connected entorhinal cortex *before* induced plaque formation. We also show that hippocampal plaques originate from axons of the entorhinal cortex concomitant with intracellular Aβ declines in connected regions; we specifically observe this in the entorhinal cortex and CA1 pyramidal layer of hippocampus. We are currently repeating these experiments in the less aggressive App^{NL-F} knock-in AD mouse model and are looking further at how seeded Aβ relates to autophagic vacuoles and lysosomes. Finally, in N2a cells, we demonstrate an equilibrium of extra- and intra-cellular Aβ that is disturbed with intracellular aggregation of Aβ (Roos *et al.*, 2021).
We show that injection of prion-like Aβ into a susceptible mouse not only accelerates plaque

formation but also affects intracellular Aβ before and with plaque induction. This has two important implications: 1) To efficiently target Aβ, the intracellular pool must also be considered. 2) The formation of an intracellular prion-like seed of Aβ likely occurs 30 years before dementia, thus treatment may need to start very early.
Roos, T.T. *et al.* (2021) 'Neuronal spreading and plaque induction of intracellular Aβ and its disruption of Aβ homeostasis', *Acta Neuropathologica*

Disclosures: **T.T. Roos:** None. **M. Garcia:** None. **G.K. Gouras:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U54 AG054349
NIH Grant R35 GM127102

Title: Degenerate hippocampal spatial mapping precedes deficits in object-location encoding and memory in the 5xFAD mouse model for Alzheimer's disease

Authors: *H. ZHANG[1], L. CHEN[1], K. G. JOHNSTON[1], J. CRAPSER[1], K. N. GREEN[1], N.-L. HA[1], A. J. TENNER[1], T. C. HOLMES[1], D. A. NITZ[2], X. XU[1];
[1]Univ. of California Irvine, Irvine, CA; [2]Univ. of California San Diego, La Jolla, CA

Abstract: A key challenge in developing diagnosis and treatments for Alzheimer's disease (AD) is to detect abnormal network activity at as early a stage as possible. In recent years, studies suggest that deficits in neural circuit activity better predict the progressive impairments of spatial memory and spatial navigation. However, the longitudinal progression of these deficits and the temporal relationship between impaired neural circuit mechanisms and memory performance in AD remain unclear. We therefore performed longitudinal *in vivo* calcium imaging on hippocampal CA1 neurons in behaving 5xFAD mice and WT controls to examine the memory and spatial representation across 4-14 months of age. AAV-CamK2a-GCaMP6f was expressed in the dorsal CA1 pyramidal neurons and neural calcium activities were recorded by miniscope when mice exploring open field, linear track or during object location memory task. 5xFAD mice show amyloid plaque accumulation at 4-month-old, while the object location memory is intact at this age. 5xFAD mice also show depressed CA1 neuronal activity during immobile states, and degenerate and unreliable hippocampal neuron spatial tuning to environmental location at 4-month-old, indicating deficits of circuit activity precedes the emergence of memory deficits. 5xFAD mice show progressive degraded spatial representation and, eventually, impaired tuning of neural activity to object-location pairings. And by 8 months of age, such degenerate circuit function is accompanied by memory deficits. Our results indicate that measurement of spatial representation and baseline firing rates in hippocampal neurons during immobile states represent

key opportunities to detect AD related abnormalities at very early stage of disease progression. The results also highlight the close connection between impaired hippocampal tuning to object locations and the presence of object location memory deficits.

Disclosures: **H. Zhang:** None. **L. Chen:** None. **K.G. Johnston:** None. **J. Crapser:** None. **K.N. Green:** None. **N. Ha:** None. **A.J. Tenner:** None. **T.C. Holmes:** None. **D.A. Nitz:** None. **X. Xu:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R01AG068215-02

Title: Sleep fragmentation leads to a sex-specific increase in sleep debt in the chimeric humanized APPxPS1 knock-in mouse model of Alzheimer's disease

Authors: *L. Z. GUO[1], A. SUBRAMONIAM[1], C. JOHNSON[2], H. WHITLOCK[3], K. KOHLER[4], T. MACHEDA[5], S. E. BARTH[6], W. BRIONES[3], K. BOSH[3], I. JOGANI[3], M. SUCHARSKI[3], M. J. DUNCAN[7], M. P. MURPHY[2], A. BACHSTETTER[5], B. F. O'HARA[1]; [1]Biol., [2]Mol. & Cell. Biochem., [3]Univ. of Kentucky, [4]Col. of Med., [5]Anat. & Neurobio., [7]Neurosci., [6]Univ. of Kentucky, Lexington, KY

Abstract: Considerable evidence supports that disruption of sleep and circadian rhythms in patients with Alzheimer's disease (AD) exacerbates neuropathology, which further impairs sleep quality. Altered sleep behavior is a leading cause of institutionalization for AD patients. The increased incidence of AD among women necessitates investigating sex differences in sleep disruption and its effects on the progression of AD pathology. Thus, we examined an APPxPS1 knock-in (KI) mouse model that better mimics AD without using transgene over expression. To assess the impact of sleep disruption on AD, we chose to focus on sleep fragmentation (SF) rather than sleep restriction, similar to the transient arousals seen in AD patients. Therefore, WT and KI female and male mice (avge 9.16 ± 1.38 mo) were either allowed to sleep undisturbed (US) or were subjected to SF consisting of sleep deprivation for 4 intervals (1 h each) evenly distributed during the light phase of the daily light-dark cycle. The US and SF treatments were conducted from Monday to Friday for 3 weeks. Sleep-wake patterns were recorded with a non-invasive PiezoSleep system, detecting vibrations that are categorized as sleep or wake at a 2sec resolution. Then, the cortex and hippocampus were dissected and Aβ40 and 42 levels were quantified in both aqueous soluble and non-soluble fractions. All SF mice had 20% less sleep compared to the US during the light phase. In the dark phase, KI females had significant rebound sleep, a 70% increase compared to its control US group ($p<0.001$). Interestingly, sleep percentage differed little across the weeks. Sleep bouts were unaffected by SF, genotype, or sex

in the light phase but varied greatly in the dark phase, with the sleep bouts of the KI females exposed to SF increasing by 49.7% compared to their US group ($p < 0.001$). SF did not affect Aβ levels in this mouse line. Aβ pathology in KI mice typically begins in the cortex, and we were able to detect this in in some fractions. A modest and marginally significant increase in Aβ was observed in females versus males in some assays. However, no other comparisons showed significant differences, perhaps due to young age of the mice. We are currently investigating Aβ in older mice to confirm this trend. Disruption of sleep during the normal rest phase led to a significant increase in sleep during the active phase, with KI females exhibiting the most robust sleep rebound. This suggests that females incur increased sleep debt during sleep disruption, which may be relevant to the increased risk and severity of AD in women.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: F31AG069496
T32AG049688
R03NS111493

Title: Entorhinal-hippocampal desynchronization in a mouse model of Alzheimer's disease pathology

Authors: *L. M. VETERE[1], L. CHEN[1], Z. CHRISTENSON WICK[1], O. LIOBIMOVA[2], D. J. CAI[1], T. SHUMAN[1];
[1]Neurosci., [2]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Background: Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by memory loss and progressive age-related cognitive decline. Impairments in hippocampal function and spatial navigation are well established in AD, but it is unclear whether this is driven by local changes or by abnormal inputs, such as those from medial entorhinal cortex (MEC). Methods: To understand how AD pathology impacts the entorhinal-hippocampal circuit, we used 3xTg mice, which express mutations in APP, presenilin, and tau. We performed acute *in vivo* silicon probe recordings to simultaneously record from hippocampus and inputs from MEC. Head-fixed mice were trained to run on a virtual reality track and recorded during active navigation. By recording in two age groups, we were able to compare neural activity

before and after the onset of spatial memory impairments and examine how and when synchrony breaks down. Results: Our data suggest that synchrony between MEC and hippocampus is impaired in 3xTg mice compared to WT controls. Decreased hippocampal theta power began to emerge by 6 months of age, prior to the onset of spatial memory impairments. However, more severe changes throughout the hippocampus were present at 8 months, when memory impairments were observed. We also found reduced theta coherence between MEC and hippocampus in 3xTg mice at 8 months, but no reduction in theta coherence between DG and CA1. Conclusions: The observed reduction in hippocampal theta power and decreased coherence between MEC and hippocampus suggest a breakdown of communication between MEC and hippocampus. Possible factors driving early entorhinal dysfunction include hyperexcitability of MECII stellate cells and loss of MEC parvalbumin interneurons. These findings align with recent studies showing early entorhinal dysfunction mouse models of AD pathology.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS094201

Title: Impact of Non-pharmacological Chronic Hypertension on a Transgenic Rat Model of Cerebral Amyloid Angiopathy

Authors: *A. STANISAVLJEVIC[1,2], J. M. SCHRADER[1,2], X. ZHU[2,1], J. M. MATTAR[1], A. HANKS[1,2], F. XU[2,1], M. MAJCHRZAK[1], J. K. ROBINSON[1,2,3], W. E. VAN NOSTRAND[2,1]; [1]George and Anne Ryan Inst. for Neurosci., [2]Dept. of Biomed. and Pharmaceut. Sci., [3]Dept. of Psychology, Univ. of Rhode Island, Kingston, RI

Abstract: Cerebral amyloid angiopathy (CAA) is a common small vessel disease characterized by deposition of fibrillar amyloid β (Aβ) in and around blood vessels of the brain causing neuroinflammation and vascular cognitive impairment and dementia (VCID), and is a frequent comorbidity of Alzheimer's disease (AD). Hypertension, another prominent small vessel disease, has been found to increase risk of dementia and may worsen associated pathologies but clinical data regarding its effects in CAA patients is controversial. For example, studies have reported that hypertension can worsen CAA related intracerebral hemorrhage (ICH), have little to no effect or even lessen CAA related ICH. To understand the effects of hypertension on pathologies of CAA, we bred rTg-DI transgenic rats, a model of CAA, with spontaneously hypertensive, stroke prone rats (SHR-SP) producing bigenic rTg-DI/SHR-SP and non-transgenic SHR-SP littermates. Also utilized in this study were rTg-DI rats and non- transgenic (WT) littermates.

Blood pressure measurements were completed at 10 months of age showing that both bigenic Tg-DI/SHR-SP and SHR-SP littermates exhibit elevated blood pressures. Subsequently, the rats were euthanized and the brain tissue was collected and assessed for known pathologies of CAA including vascular amyloid load, small vessel occlusions, microbleeds, and perivascular glial cell activation. Vascular Aβ load of hippocampus and thalamus was significantly decreased in bigenic rTg-DI/SHR-SP rats compared to rTg-DI rats. On the other hand, CAA load of small pial vessels was significantly increased in bigenic rats compared to rTg-DI rats. No difference in microbleeds was observed between SHR-SP, rTg-DI, and bigenic rTg-DI/SHR-SP animals. Though total number of thalamic small vessel occlusions were no different between rTg-DI and bigenic rTg-DI/SHR-SP rats, the size and distribution pattern were significantly altered in bigenic rats. Additionally, an enlargement of cortical perivascular space observed in SHR-SP rats was adopted by bigenic rats. Proteomic analysis of cortical tissue showed that rTg-DI/SHR-SP rats exhibited a similar signature to rTg-DI rats, but with some enhancements. Our findings show that non-pharmacological hypertension in 10 month rTg-DI rats causes a redistribution of vascular CAA along with a significant change in size and distribution of thalamic small vessel occlusions. This study presents a non-pharmacological model to further investigate hypertension and CAA as co-morbidities for cerebral small vessel disease.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1RF1AG062234-01

Title: Machine Learning Approaches Reveal Prominent Behavioral Alterations and Cognitive Dysfunction in a Humanized Alzheimer Model

Authors: *S. R. MILLER[1], K. LUXEM[3], N. KALISS[1], Y. QIU[1], P. NAMBIAR[1], C. CAI[1], K. SHEN[1], T. SAITO[4], T. C. SAIDO[5], A. PICO[2], R. THOMAS[2], S. REMY[6], J. J. PALOP[1]; [1]Gladstone Inst. of Neurolog. Dis., [2]Gladstone Bioinformatics Core, Gladstone Inst., San Francisco, CA; [3]Univ. of Bonn, Bonn, Germany; [4]RIKEN, Wako, Japan; [5]RIKEN Brain Sci. Inst., Saitama, Japan; [6]Neuronal Networks Group, DZNE German Ctr. For Neurodegenerative Dis., Bonn, Germany

Abstract: Behavioral manifestations define neurological disorders, but our knowledge of disease induced behavioral alterations is incomplete and largely limited to standard domain-specific behavioral approaches. Newly humanized App knock-in (KI) models of Alzheimer's disease

(AD) mimic disease mechanisms better than transgenic overexpression models, but fail to display consistent behavioral alterations in standard behavioral tests despite severe AD-related neuropathological changes. To address this technological barrier in AD modeling, we used machine learning platforms to test the hypothesis that deconstructing full sequences of spontaneous mouse behavior into canonical behavioral units (motifs) will better capture AD-induced brain dysfunction in App-KI mice. Indeed, we found that humanized $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice have robust impairments in spontaneous behavior evidenced by prominent alterations in motif use and motif transitions consistent with cognitive dysfunction, including blunted novelty response, impaired habituation and sensitization, and disorganized behavioral sequences. We conclude machine learning approaches provide a direct and unbiased measure of AD-related mechanisms of brain dysfunction.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

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Program #/Poster #: 616.19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Louis and Harold Price Foundation
H & H Evergreen Foundation
Jonathan and Susan Dolgen Foundation

Title: Supervised machine learning reveals the heterogeneous changes of gait kinematics in response to β amyloid level alteration in a mouse model of neurodegeneration

Authors: *R. HUANG[1,2,3], M. RASOOLINEJAD[1], H. ZENG[1], H. YANG[1], J. C. LEITER[1,5], D. C. LU[4,2,3];
[1]Univ. of California Los Angeles, Los Angeles, CA; [2]Brain Res. Inst., [3]Neurosurg., [4]UCLA, Los Angeles, CA; [5]Dartmouth Col., Hanover, NH

Abstract: Motor deficits are observed in Alzheimer's disease (AD) prior to the appearance of cognitive symptoms. Slower stepping speed, as well as the imbalanced gaits, have been reported in patients with AD as well as the individuals with mild dementia who were later diagnosed with AD. The underlying mechanism of how these gait disturbances are induced by AD progression remains unknown. Our previous study proposed an analytical pipeline based on machine learning to characterise locomotion in amyloid precursor protein (APP)-overexpressing transgenic J20 mice. We used three-dimensional motion capture to characterize quadrupedal locomotion on a treadmill in J20 and wild-type mice. The genotypes of the animals were classified with average accuracy rates of $92.3 \pm 5.2\%$ and $93.3 \pm 4.5\%$ and a False Negative Rate (FNR) of $0.0 \pm 0.0\%$

and $0.0 \pm 0.0\%$ for the 4-month and 13-month groups of mice, respectively. However, the evidence supporting the role of APP and its proteolytic products in gait disturbances is still lacking. Therefore, we tested the hypothesis that the gait disturbance in J20 mice could be corrected by inhibiting β amyloid generation using a gamma-secretase inhibitor (GSI). We also hypothesize that the kinematic features that contributed to classifying the gait changes induced by GSI treatment, as defined using machine learning, could potentially reveal the neuromuscular mechanism(s) underlying the gait changes uniquely correlated to β amyloid. We trained our model with the kinematics features captured from 18 4-month (4mon) J20 mice, 14 4mon WT mice, 14 10-month (10mon) J20 mice, and 9 10mon WT mice before GSI treatment. Next, we delivered GSI (Benzodiazepine-type LY-411575, oral gavage daily for four consecutive days) to all the animals and collected kinematic features of quadrupedal treadmill stepping after 4 days of GSI treatment. GSI treatment significantly increased the maximum running speed in 4mon only but not in 10mon J20 mice (4mon J20 GSI vs no GSI: $p = 0.0016$; 10mon J20 GSI vs no GSI: $p = 0.1822$) on the treadmill and significantly decreased the number of steps during which a foot was dragged (4mon J20 GSI vs no GSI: $p < 0.0001$; 10mon J20 GSI vs no GSI: $p < 0.0001$) in both 4mon and 10mon J20 mice. We used a random-forest classification model trained on a no GSI treatment, kinematics dataset, but failed to predict the genotype of the animals with GSI treatment for both WT and J20 mice. This inconsistency between the computational prediction based on kinematics features and the motor behavioural results suggests that GSI may negatively impact quadrupedal kinematics features unrelated to the maximum stepping speed and the steps with drag.

Disclosures: **R. Huang:** A. Employment/Salary (full or part-time):; University of California, Los Angeles. **M. Rasoolinejad:** None. **H. Zeng:** None. **H. Yang:** None. **J.C. Leiter:** A. Employment/Salary (full or part-time):; University of California, Los Angeles, Dartmouth College. F. Consulting Fees (e.g., advisory boards); MAXIS LLC. **D.C. Lu:** A. Employment/Salary (full or part-time):; University of California, Los Angeles. 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.; Jonathan and Susan Dolgen Foundation, The Louis and Harold Price Foundation, H & H Evergreen Foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Restore Technologies, Inc..

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.20

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH R01 AG064902
NIH P50 AG005681

NIH P01 NS074969
Alzheimer's Association

Title: Beta-arrestin involvement in differential effects of acute stress on Abeta and tau levels in male and female mice

Authors: *J. CIRRITO, H. M. EDWARDS, C. M. YUEDE, W. D. GARDINER; Washington University, St. Louis, Saint Louis, MO

Abstract: Almost 70% of people living with AD are female. Interestingly, stress-induced corticotrophin releasing factor receptors (CRF-Rs) signal differently in females and males. In females, CRF-Rs normally activate PKA/ERK. In males, CRF-Rs are withdrawn from the plasma membrane by beta-arrestin, resulting in significantly less CRF signaling. We hypothesize that the involvement of beta-arrestin in the stress signaling pathway in males underlies the differences in abeta levels in response to stress. We used in vivo microdialysis to measure brain ISF Abeta levels every hour for several hours before, during, and after acute restraint stress in living APP transgenic mice. To study the influence of beta-arrestin1 on stress-induced changes in Abeta, we measured the effects of acute stress on Aβ levels in male and female beta-arrestin1 knock-out mice. To elucidate the CRF-signaling pathways, we used CRF, PKA, and ERK inhibitors before acute stress exposure. In females, acute restraint stress causes a rapid increase in brain interstitial fluid (ISF) Aβ levels in the hippocampus, whereas Aβ in males does not change. The increase in females is blocked by inhibiting the CRF receptor (CRF-R), PKA and ERK pathways. In male beta-arrestin1 knockout mice, stress increases ISF Aβ levels nearly identically to females. Our data suggest that stress causes sex-dependent increases on Aβ and that are mediated by CRF-R/beta-arrestin signaling. Determining the cellular pathways that differ between the sexes could identify risks of developing AD and lead to therapeutics to specifically modulate the stress response in AD, potentially that vary by sex.

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Poster

616. APP/Abeta Cellular and Animal Models I

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH UL1 TR002535
NIH R03 AG072102
Boettcher Foundation

Title: Co-activation of selective nicotinic acetylcholine receptors improves hippocampal brain rhythms and memory in the mouse of Alzheimer's disease.

Authors: *R. LEE, S. KIM;
Colorado State Univ., Colorado State Univ., Fort Collins, CO

Abstract: It has been suggested that reduced activity in GABAergic inhibitory interneurons disrupts neural oscillations in the hippocampus, which leads to memory loss in Alzheimer's disease (AD). A prominent AD pathology in the human brain is the loss of cholinergic neurons and nicotinic acetylcholine receptors (nAChR). Aβ is known to interact with these receptors and impair their function. nAChRs are expressed more in GABAergic inhibitory interneurons, thus cholinergic deficiency is a prime suspect for Aβ-induced impairment of inhibitory dysfunction in the hippocampus and cognitive decline in AD. Our previous findings, using cultured mouse hippocampal neurons show Aβ selectively interacts with α7- and α4β2-nAChRs, but not α3β4-nAChRs, and decreases activity in inhibitory interneurons, but induces hyperexcitation in excitatory neurons. We thus hypothesize that Aβ reduces hippocampal GABAergic activity by selectively inhibiting α7- and α4β2-nAChRs, resulting in hippocampal oscillatory disruption and memory loss in AD. To test our hypothesis, the AD mouse model, 5XFAD transgenic mice, and wild type (WT) littermates were treated intraperitoneally with α7- and α4β2-nAChR agonists. Saline was given to control mice. Fear conditioning was performed to see if agonists improved memory. We found that 5XFAD mice showed clear deficit in contextual memory which was successfully reversed by co-stimulation of α7- and α4β2-nAChR agonists. Stereotaxic surgery was then performed to measure local field potentials of theta and gamma oscillations, key components for learning and memory. During memory consolidation, theta, slow and fast gamma activities were significantly reduced in 5XFAD mice. Co-activation of α7- and α4β2-nAChR agonists was sufficient to restore normal rhythmic activities in 5XFAD mice. Ca^{2+} imaging with nicotine uncaging further reveals that cultured hippocampal neurons expressed α7- and α4β2-nAChRs in parvalbumin-positive (PV+) and somatostatin-positive (SST+) cells, respectively. These two major types of GABAergic inhibitory interneurons play key roles in hippocampal network activity and learning and memory. Thus, co-activation of the two receptors is important for restoring hippocampal activity and memory in 5XFAD mice.

Disclosures: **R. Lee:** None. **S. Kim:** None.

Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.22

Topic: C.02. Alzheimer’s Disease and Other Dementias

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College Research Council Shared Research Program from Colorado State University
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the University of Hawai'i Foundation Preclinical Alzheimer's Research Fund

Title: Selective co-activation of α7- and α4β2-nicotinic acetylcholine receptors reverses beta-amyloid-induced synaptic dysfunction

Authors: J. P. ROBERTS[1], S. A. STOKOE[1], M. F. SATHLER[1], R. A. NICHOLS[2], *S. KIM[1]; [1]Colorado State Univ., Colorado State Univ., Fort Collins, CO; [2]Univ. of Hawaii, Honolulu, HI

Abstract: Beta-amyloid (Aβ) has been recognized as an early trigger in the pathogenesis of Alzheimer's disease (AD) leading to synaptic and cognitive impairments. Aβ can alter neuronal signaling through interactions with nicotinic acetylcholine receptors (nAChRs), contributing to synaptic dysfunction in AD. The three major nAChR subtypes in the hippocampus are composed of α7-, α4β2-, and α3β4-nAChRs. Aβ selectively affects α7- and α4β2-nAChRs, but not α3β4-nAChRs in hippocampal neurons, resulting in neuronal hyperexcitation. However, how nAChR subtype selectivity for Aβ affects synaptic function in AD is not completely understood. Here, we showed that Aβ associated with α7- and α4β2-nAChRs but not α3β4-nAChRs. Computational modeling suggested two amino acids in α7-nAChRs, arginine 208 and glutamate 211, were important for the interaction between Aβ and α7-containing nAChRs. These residues are conserved only in the α7 and α4 subunits. We therefore mutated these amino acids in α7-containing nAChRs to mimic the α3 subunit and found that mutant α7-containing receptors were unable to interact with Aβ. Additionally, mutant α3-containing nAChRs mimicking the α7 subunit interact with Aβ. This provides direct molecular evidence for how Aβ selectively interacted with α7- and α4β2-nAChRs, but not α3β4-nAChRs. Selective co-activation of α7- and α4β2-nAChRs also sufficiently reversed Aβ-induced AMPA receptor (AMPAR) dysfunction, including Aβ-induced reduction of AMPAR phosphorylation and surface expression in hippocampal neurons. Moreover, co-stimulation of α7- and α4β2-nAChRs reversed the Aβ-induced disruption of long-term potentiation. These findings support a novel mechanism for Aβ's impact on synaptic function in AD, namely the differential regulation of nAChR subtypes.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Nature Science Foundation of China 91849125
Nature Science Foundation of China 31571048

Title: Sex difference in β-amyloid in triple-transgenic AD mice and the role of estrogen in protecting EGR1 from soluble Aβ42 inhibition

Authors: *Y.-T. HU[1], X.-L. CHEN[1], Y.-N. ZHANG[1], H. MCGURRAN[2], N. BREEUWSMA[2], D. SWAAB[2], A.-M. BAO[1];
[1]Zhejiang Univ. Sch. of Med., Hangzhou, China; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Deposition of β-amyloid (Aβ) may occur decades before Alzheimer's Disease (AD) diagnosis. The reduction of estrogen has been proposed to play a key role in explaining women's higher risk of developing AD than men, while the mechanism remains misty. Early growth response-1 (EGR1) is involved in Aβ production, and the increase of EGR1 in early AD is involved in keeping cholinergic function intact. We aim to investigate whether Aβ deposition shows sex difference in the triple-transgenic (3xTg) AD mice, and whether and how estrogen regulates Aβ-related EGR1 expression in vitro. 3xTg-AD mice were sacrificed at three different months of age, half male and half female in each age group. In the hippocampal complexes (HpC), we measured Aβ levels by immunohistochemistry and ELISA, and EGR1 levels by qPCR. In addition, SY5Y cells were infected with lentivirus containing the amyloid precursor protein construct C99. By with and without dosing 17β-estradiol (E2) into the culture medium, we analysed extracellular Aβ levels by ELISA and intracellular EGR1 levels by qPCR and Western Blot. In the mouse study, 1) higher Aβ levels were found in the subiculum of HpC in females than males for the entire group (P=0.02); 2) only at 11-12 months of age, cored Aβ plaques appeared in the subiculum of females but not males; 3) females of 3-4 months had lower ($P\leq$0.04) while those of 11-12 months had higher ($P\leq$0.03) soluble Aβ40 and Aβ42 levels than their age-matched males; 4) females of both 7-8 and 11-12 months ($P\leq$0.04 and P<0.001) presented higher insoluble Aβ40 and Aβ42 than their age-matched males. Although EGR1 showed no sex difference in each age group, it was negatively correlated with soluble Aβ42 only in males (r=-0.57, P=0.01). In the cell line study, 1) increased Aβ42 (P=0.02) and decreased EGR1 levels (P<0.001) were found in CT99-SY5Y cells compared with control cells; 2) reduced Aβ42 (P=0.01) and elevated EGR1 levels (P<0.001) were observed in CT99-SY5Y cells cultured in E2-containing medium compared to cells in E2-free medium. Aβ40 levels did not vary between groups. Aβ levels were higher and accumulated faster in the subiculum in female 3xTg-AD mice, and the negative correlation between soluble Aβ42 and EGR1 presented only in males suggested a possible role of estrogen in protecting EGR1 from being inhibited by soluble Aβ42. The SY5Y cell line findings supported the inhibitory effect of Aβ42 on EGR1 and that E2 can restore EGR1 levels by reducing Aβ42.

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Poster

616. APP/Abeta Cellular and Animal Models I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 616.24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG057767
R01AG061937
The Dale and Deborah Smith Center for Alzheimer's Research and Treatment
Kenneth Stark Endowment

Title: Sex and Age-Based Differences in the APP/PS1 and $APP^{NL-F/NL-F}$ Models of Alzheimer's Disease

Authors: *S. PANDEY, K. QUINN, M. PECK, S. MCFADDEN, C. FINDLEY, K. HASCUP, E. R. HASCUP;
SIU Sch. of Med., Springfield, IL

Abstract: Alzheimer's disease (AD) is an age-related neurological disorder typically associated with diminished cognitive function and memory loss that interferes with the ability to perform activities of daily living. In this study, cognitive, metabolic, and physical parameters were assessed in male and female transgenic (APP/PS1) and knock-in ($APP^{NL-F/NL-F}$ and $APP^{NL/NL}$) mouse models of AD along with C57BL/6 littermate controls. All mice were evaluated at 8-10 (early amyloid plaque deposition) or 21-23 (severe amyloid plaque deposition) months of age. Cognitive performance was assessed by novel object recognition (NOR), Y maze, and Morris water maze (MWM). In the 8-10 month groups, no deficits were observed in NOR retention index or Y maze spontaneous alternations. On training day 2 of MWM, APP/PS1 females navigated significantly further from the platform compared to C57BL/6 females and $APP^{NL-F/NL-F}$ females. On training days 2 and 3, $APP^{NL-F/NL-F}$ females spent significantly less time in the target quadrant than $APP^{NL/NL}$ females, however on training day 5, the difference was not significant. For long-term memory measures, $APP^{NL-F/NL-F}$ females showed a decreased trend in the number of platform and annulus-40 entries compared to C57BL/6 females during MWM. No differences were observed in male mice. In the 21-23 month group, no differences were observed in NOR retention index regardless of genotype or sex. No deficits were seen in Y maze for transgenic and knock-in models of either sex. On MWM training days 4 and 5, male APP/PS1 mice navigated further from the platform compared to $APP^{NL/NL}$ and C57BL/6 males. APP/PS1 males also spent significantly less time in the target quadrant than C57BL/6 males on training days 2-5. On training day 5, female APP/PS1 and $APP^{NL-F/NL-F}$ navigated significantly further from platform than C57BL/6 females. On training day 5, $APP^{NL-F/NL-F}$ females, spent significantly less time in the target quadrant than C57BL/6 females. No significant genotype differences were found in either sex in the long-term memory measures. The analysis of metabolic and physical parameters is ongoing. These preliminary findings support a trend of sex-based differences in spatial memory tasks in different age groups in the AD mouse models.

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Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 617.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA/NIH R01AG57931
NIA/NIH RF1AG059093

Title: Characterization of cognitive function and amyloid beta in a humanized APOE Alzheimer's disease mouse model

Authors: *J. ALTEMUS, H. CORTES-FLORES, J. P. WIEGAND, R. D. BRINTON; Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: The apolipoprotein E gene variant ε4 (APOEε4) is the greatest genetic risk factor for late onset Alzheimer's disease (LOAD). The purpose of this study was to assess the translational validity of the humanized (h) APOE genetic mouse model for APOEε4 (JAX #027894) and APOEε3 (JAX #029018) (N=8-12 mice / group). Analyses of the hallmark pathologies of cognitive decline and β-amyloid (Aβ) load in the brain and periphery were conducted. Male and female 18-month old homozygous hAPOEε3/3, homozygous hAPOEε4/4, or heterozygous hAPOEε3/4 underwent tests of novel object recognition (NOR) cognition, open field (OF) anxiety and a range of motor functions. NOR measures included discrimination index and combined object exploration time, OF included center zone time and speed, and motor function included cadence and speed. Aβ38, Aβ40, and Aβ42 oligomers in plasma and hippocampus levels in the same cohort of mice were determined by Mesoscale Discovery (MSD) ELISA. A sex difference was seen in plasma amyloid levels with females having lower Aβ40, Aβ42, and Aβ42/40, specifically hAPOEε3/3 females when analyzed by genotype + sex. The sex difference was not evident in hippocampal amyloid levels, indicating that the role of sex in modulating amyloid levels may be specific to peripheral effects. Interestingly, hippocampal Aβ42 was lower in hAPOEε3/4 mice vs hAPOEε3/3 and hAPOEε4/4 mice, suggesting a potential neuroprotective effect in heterozygous mice. Hippocampal Aβ40 and Aβ42/40 ratios were not different across genotype or sex, and Aβ38 was not detected in any group. OF mean speed was lower in hAPOEε3/4 mice vs hAPOEε4/4 mice, while OF center zone time showed no differences across groups. NOR analyses and motor function analyses indicated no differences across genotype or sex. Male vs female analyses were performed using two-sided t-tests, while all other analyses were performed using one way ANOVA. These differential peripheral and brain Aβ levels across genotype and sex suggest potential translational applicability in 18-month (human age equivalent ~56 years) APOE mice. This age point may be too early to detect cognitive and motor function changes in this mouse model.

Disclosures: J. Altemus: None. **H. Cortes-Flores:** None. **J.P. Wiegand:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant P01AG026572
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The Women's Alzheimer's Movement
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Title: Change in white matter integrity across the menopausal transition in women and in relation to age-matched men

Authors: *A. C. RAIKES[1], J. P. DYKE[2], S. JETT[3], E. SCHELBAUM[3], S. PAHLAJANI[3], R. D. BRINTON[1], L. MOSCONI[2,3];
[1]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ; [2]Dept. of Radiology, [3]Dept. of Neurol., Weill Cornell Med., New York, NY

Abstract: Women develop Alzheimer's disease (AD) at a 2:1 rate compared to men. Changes associated with menopause exacerbate a midlife bioenergetic crisis that, when unsuccessfully resolved, leads to white matter degradation and ultimately brain atrophy. Changes in brain structure across the menopausal transition during mid-life could have predictive value for determining AD risk in later life. Here, we report differences in white matter integrity between pre- (n=40, 44.1±3.34y), peri- (n=60, 49.6±3.90y), and post-menopausal women (n=70, 56.0±4.12y), as well as comparisons with comparably aged males (n=46, 50.8±7.50y). Diffusion weighted MRI was acquired for each participant. Data were preprocessed with QSIPrep (v. 0.15.3) and scalar maps were produced using DIPY (v. 1.5.1). Average fractional anisotropy (FA), mean (MD), axial (AD), and radial diffusivity (RD) were computed in 50 white matter ROIs. These scalar values were adjusted for nonlinear age effects (3rd order polynomial) and global diffusivity value in white matter voxels. Between-group differences were computed as Cohen's d, 95% bootstrapped confidence intervals and empirical p-values. Significant findings are defined where confidence intervals exclude 0 and FDR-adjusted $p < 0.05$. Few diffusion scalar differences were observed between pre-, peri-, and post-menopausal women. Greater FA and lower RD were observed in the genu of the corpus callosum for peri- compared to pre-menopausal women. Compared to pre-menopausal women, post-menopausal women had greater FA (d=0.51) and AD (d=0.40) in the left uncinate fasciculus and left corticospinal tract respectively. Further post-menopausal women had greater FA and MD in the right inferior cerebellar peduncle (d=0.36) and left medial lemniscus (d=0.37) respectively than peri-menopausal women. By contrast, lower FA and greater MD and RD in women compared to the men was observed in 24/50 regions, including the bilateral hippocampal cingulum, corpus callosum, and bilateral internal and external capsules. These differences were moderate to large ($0.66<|d|<1.10$). Few white matter differences were evident in women relative to menopausal transition state However greater isotropic diffusion in white matter compared to comparably aged males suggests the potential for early, persistent changes in white matter integrity

unexplained by age or global diffusivity. These findings may provide evidence of supporting evidence for the greater risk of AD in women versus men.

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Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 617.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P01AG026572-Project 1
NIH Grant P01AG026572-Analytic Core
NIH Grant RF1AG068175

Title: Apoe4 augments metabolic-inflammatory transformations in glial cells during perimenopausal transition

Authors: *Y. MI[1], G. QI[2], T. WANG[3], R. D. BRINTON[4], F. YIN[5];
[1]Univ. of Arizona, Tucson, AZ; [2]The Ctr. for Innovation in Brain Sci., Univ. of Arizona, TUCSON, AZ; [3]Ctr. for Innovation in Brian Sci., [4]Ctr. for Innov in Brain Sci., [5]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Perimenopause is a neuroendocrine transition state unique to females. Our previous research in both human and rodent models revealed that perimenopause is neurologically a critical transition period characterized by bioenergetic- and neuroinflammatory changes that are reminiscent of prodromal Alzheimer's disease (AD). As the greatest genetic risk factor of AD, ApoE4 adversely affects women much greater than men. However, the mechanisms by which ApoE4 and the perimenopausal transition converge to disrupt neural functions remain to be explored. The specialized roles neurons and glia play across the metabolic-inflammatory systems and recent advances in single-cell analyses of AD brains emphasize the importance of devising cell-type-specific approaches to investigating mechanisms underlying perimenopause- and ApoE4 driven transformations. We therefore performed single-nucleus RNA-seq (snRNA-seq) analysis in our Perimenopausal Animal Model (PAM) with different endocrine status and ApoE genotype. Our analysis of female hApoE3 and hApoE4 mice at premenoapuse (9-month regular cycling) or perimenopause (15-month Irregular cycling) detected and distinguished major brain cell types with an average of 21,595 transcripts and 2,226 genes per unique nucleus. An increase in astrocytes and a decrease in excitatory neurons were observed in both Irreg-15M ApoE3 and ApoE4 groups compared to their Reg-9M counterparts and, importantly, these changes in cellular composition were exacerbated by ApoE4 at 15M. Further, transcripts of genes involved

in astrocyte reactivity were elevated in astrocytes of Irreg 15M brains, with a further upregulation in the ApoE4 Irreg 15M group, which was accompanied by alterations in metabolic genes in astrocytes, particularly those involved in lipid metabolism. Further, relative to microglia of the 9M brains and the ApoE3 Irreg-15M brains, ApoE4 Irreg-15M microglia exhibited the highest expression of proinflammatory gene. Collectively, these findings suggest that a shift in lipid metabolism in tandem with activated glia and proinflammatory profile is promoted by both perimenopause transition and ApoE4, which could underlie the early disruption to lipid homeostasis in Alzheimer's brains and the ApoE4- and female sex-associated elevation in Alzheimer's risks. This work has been supported by the National Institute on Aging (NIA) grants P01AG026572 to RDB (Project 1 and Analytic Core to FY, Animal Core to TW) and RF1AG068175 to FY.

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Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R37AG053589

Title: Effects of estrogen receptors on mitochondrial morphology and function in a neuronal cell line

Authors: *M. PADILLA - RODRIGUEZ[1], J. ALTEMUS[1], T. WANG[2], H. CORTES-FLORES[3], W. J. MCLEAN[1], G. L. BRANIGAN[5], R. D. BRINTON[4];
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Abstract: Estrogens are an important steroid hormone, involved in maintaining the integrity of several tissue systems including the immune system, cardiovascular system, central nervous system, and brain development. During the female lifecycle, estrogen levels fluctuate with the estrous cycle and pregnancy, ultimately declining with age. This decrease in estrogen signaling has several important effects on tissues sensitive to estrogen, most notably the brain. While estrogen receptors (ERs) play several roles in the brain, many of its functions converge on cellular metabolism and bioenergetics, and suggests that a decline in estrogen levels with age may disrupt these pathways. In this study, we investigated how altering ER expression, specifically the nuclear ERs (ERα and ERβ) and the membrane-embedded ER, GPER1, affect bioenergetics and cellular metabolism. Using a human neuronal cell line, we generated ERα, ERβ, and GPER1 KO lines by CRISPR/Cas9 to determine the role of ER activity on mitochondrial function and morphology. Quantification of mitochondrial respiration using Agilent Seahorse XF Analyzer showed that basal respiration, maximal respiration, ATP

production were not significantly different between WT and ERα KO cells. However, basal respiration, maximal respiration and ATP production were significantly lower in ERβ KOs and GPER1 KOs compared to WT cells. Results from immunofluorescent staining showed that ERα KOs had significantly smaller cell bodies compared to WT, while ERβ and GPER1 KO cells exhibited a large, flat cell morphology. ERβ and GPER1 KO cells also showed a distinct shift in the distribution of mitochondrial morphology, from small punctate and tubular mitochondria common in WT cells, to large mitochondrial networks. These observations were further confirmed by transmission electron microscopy, showing that ERα KO mitochondria were qualitatively smaller and more punctate than WT, whereas ERβ KO and GPER 1 KO mitochondria were larger and more elongated. Together, these data suggest ERs, specifically ERβ and GPER1, play an important role regulating cell morphology, mitochondrial function and morphology in human neuronal cells.

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Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant 1T32AG061897
NIH/NIA Grant R01AG057931

Title: Prodromal neuroimmune and metabolic reprogramming are accelerated and sustained in the female hAPOE ε4/4 mouse brain

Authors: *N. DELATORRE, H. VAN ROSSUM, A. MISHRA, M. PADILLA-RODRIGUEZ, Y. SHANG, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Age, female sex, and *APOE ε4* are prominent risk factors for Alzheimer's Disease (AD) with *APOE ε4* carrying women having the highest risk for development of AD in comparison to men. Neuroinflammation is a well-documented feature of AD that is known to be impacted by the above risk factors. Previous lab findings indicated increased inflammatory profile and a metabolic change in female h*APOE ε4* mouse brain relative to age-matched males. We hypothesize that the energy shift at midlife creates a prodromal condition leading to increased microglial activity in the white matter regions of the aged hAPOE mouse brains.18-month-old humanized *APOE ε3/3, APOE ε3/4,* & *APOE ε4/4*, male and female mice from Jackson labs were used. Brains were perfused and fixed for immunohistochemistry (IHC).

Several markers were used for IHC; microglia identifier (IBA1), activation markers (MHCII & IBA1), and phagocytosis marker (CD68). Hippocampal tissues were sent for transcriptomic analyses to investigate neuroinflammatory and bioenergetic markers.
Cell phenotyping analysis indicated an *APOE* genotype difference in microglial activation in the corpus callosum (CC). Female h*APOE ε4/4* exhibited increased microglial activity indicated by increased MHCII, CD68, IBA1 fluorescence intensity (FI), and decreased microglial density in comparison to the female h*APOE ε3/3*. The transcriptomic relative analysis score indicated a similar trend in the inflammatory response pathway with h*APOE ε4/4* females exhibiting greater response relative to h*APOE ε3/3*. Transcriptomic analysis also revealed h*APOE ε4/4* mice have a decrease in the overall bioenergetic pathway (TCA) with a sex driven fuel source preference. Females exhibited greater fatty acid beta oxidase pathway expression while males exhibited greater glycolysis pathway gene expression. Transcriptomic PCA analysis plot PC2 showed an absolute separation by sex in all ages. At 12m we can see a clear separation in plot PC3 associated to *APOE* genotype in the females. The same separation can be seen in the 18m males.These results further support our hypothesis that sex differences in the risk of late-onset AD are detectable at the prodromal phase of AD. Neuroimmune activation and metabolic reprogramming that is initiated during the prodromal phase is sustained in later life. *APOE ε4* has an accelerating effect with sex and genotype differences exhibiting earlier in females relative to the same genotype males.

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Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 617.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG057931
R01AG057931-02S1
S10OD025016

Title: Studying the impact of bioenergetic shift on hippocampal volume using behavioral clustering in a humanized APOE mice model for Alzheimer's Disease

Authors: *A. BHATTRAI[1], A. RAIKES[2], J. W. MCLEAN[2], F. VITALI[2], J.-P. WEIGAND[2], R. D. BRINTON[2];
[1]Pharmacol., [2]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Apolipoprotein (APOE) ε4 is the strongest genetic risk factor for the development of late-onset Alzheimer's disease (LOAD). Clinically, LOAD exhibits strong sex effects with females having a 2x greater risk of developing AD than males. MRI studies have shown a link between APOE-ε4 gene load and reduced hippocampal volume as well as accelerated hippocampal volume loss. This study explores the effects of sex on the brain volumetric changes in aged humanized *APOE* (hAPOE) mouse model for LOAD. Two distinct behavioral clusters were identified (Cluster 1: increased activity, equal distribution of *ε4* carriers and non-carriers; Cluster 2: less active, predominantly *ε4* carriers). In a subset of the above data (N = 42; mean age= 24.1$\pm$0.632), high-resolution ex-vivo MRI was used to quantify atlas-based regional volumes. These volumes were corrected for age and total brain volume prior to analyses. Two-way ANOVAs were used to identify cluster and sex interaction effects on left and right hippocampal volume separately. Given significant interaction effects, post-hoc within-cluster sex differences and within-sex cluster differences were empirically determined using a bootstrapping procedure (n=20000 bootstraps) to compute Hedges' g effect sizes and 95% confidence intervals as well as permutation-based (n=5000 permutations) between-groups p-values. P-values were adjusted for multiple comparisons for each hippocampus separately. Significant interaction effects were observed for the left ($F_{1,38} = 6.470$, $p = 0.015$) and right ($F_{1,38} = 9.331$, $p = 0.004$) hippocampi. Post-hoc testing revealed that males in Cluster 1 had larger hippocampi than Cluster 1 females (Left: $g = 1.664$, 95% CI: 0.493-2.866, $p_{Holm} = 0.0058$; Right: $g = 1.411$, 95% CI: 0.480-2.284, $p_{Holm} = 0.0142$) as well as males in Cluster 2 (Left: $g = 1.561$, 95% CI: 0.688-2.437, $p_{Holm} = 0.0043$; Right: $g = 1.801$, 95% CI: 0.0.728-2.677, $p_{Holm} < 0.0001$). No other differences were identified. Overall, hippocampal volume was greater in Cluster 1 compared to Cluster 2, though this was driven by the males as noted above (Left: $g = 0.991$, 95% CI: 0.216-1.716, $p = 0.0016$; Right: $g = 1.313$, 95% CI: 0.521-1.927, $p_{Holm} < 0.0001$). Aged *h*APOE-ε4 mice with lower activity exhibited lower hippocampal volume compared to those with higher activity, mimicking findings from human populations. Male non-carriers were the key driver of the differences, further supporting clinical data identifying female ε4-carriers to be at the highest risk. These findings may be indicative of adverse bioenergetic adaptations in the lower-performing cluster, resulting in decreased hippocampal volume and ultimately poorer cognitive function.

Disclosures: **A. Bhattrai:** None. **A. Raikes:** None. **J.W. McLean:** None. **F. Vitali:** None. **J. Weigand:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P01AG026572
NIH Grant T32AG061897

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Women's Alzheimer's Movement
Center for Innovation in Brain Science

Title: Trajectories of resilient versus susceptible aging JAX humanized APOE by sex and weight

Authors: *F. VITALI[1], J.-P. WIEGAND[1], A. TUCKER[2], R. D. BRINTON[1];
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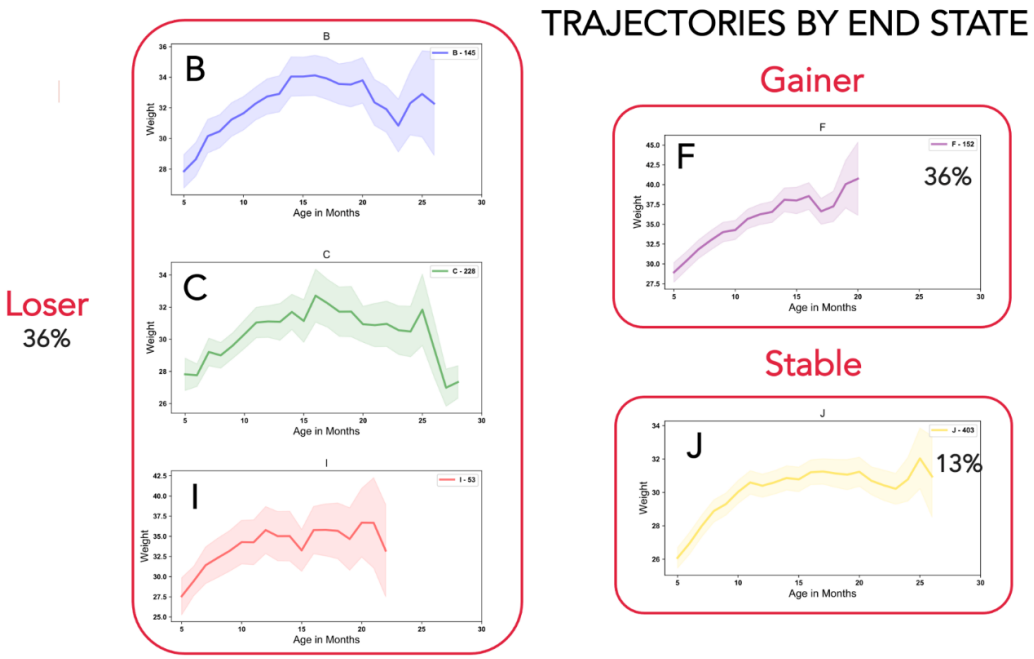
Abstract: The translational potential of JAX humanized APOE (hAPOE) mouse models for Alzheimer's disease (AD) remains unsolved. Aging, chromosomal sex, and genetic risk factors interact to determine the trajectory of an individual's age-related biological changes and in turn may predispose individual risk or resilience to AD. In literature, there is growing evidence of inexplicable and accelerated weight loss prior to AD diagnosis. In support with growing evidence, we hypothesize that weight trajectories in our JAX humanized APOE mice cohort can be an early indicator of AD disease trajectory.
We used an autoregressive hidden Markov model (AHMM) to identify trajectories of JAX humanized APOE mice based on their longitudinal weight data. We assumed 10 hidden states to estimate proper trajectories. We ran 10 AHMM models to ensure stability and each model was run with 1500 iterations to reach convergence. The AHMM with maximum trace value was selected as the main model. The key trajectories were then identified by clustering together mice ending in the same hidden state. Weight data were collected from November 2018 to September 2021 for 1196 hAPOE mice (652M, 544F) for at least 3 time points ranging from 5 to 28 months of age. Genotypes included APOE3/3, APOE3/4 and APOE4/4.
AHMM results identified 5 main weight trajectories for mice ending in one of the 5 most probable ending states (Figure1). Of these, three states captured weight loss trajectories for 426 (36%) mice, one state was characterized by an increase in weight for 152 (36%) mice and 403 (13%) mice ending in a state stable with no significant weight loss or increase. The weight loss is more apparent in female, while weight increase is more typical of male mice.
This mouse cohort displays weight loss that may correspond to individuals exhibiting a hallmark inexplicable weight loss starting around 15/20 months (corresponding to human 50/55 years old) that can be symptomatic of the prodromal phase of AD.



Disclosures: **F. Vitali:** None. **J. Wiegand:** None. **A. Tucker:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 617.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P01AG026572
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NIH Grant P01AG026572

Title: Association Between Type 2 Diabetes Therapeutics and Risk of Developing Alzheimer's Disease and Multiple Sclerosis

Authors: *G. TORRANDELL[1], G. L. BRANIGAN[4], K. E. RODGERS[2], R. D. BRINTON[3]; [1]Univ. of Arizona - Tucson, AZ, [3]Univ. of Arizona, [2]Univ. of Arizona, Tucson, AZ; [4]Univ. of Arizona Hlth. Sci., Univ. of Arizona Hlth. Sci., Tucson, AZ

Abstract: The association between use of anti-hyperglycemic medication (A-HgM) for type 2 diabetes (T2D) treatment and incidence of Alzheimer's Disease (AD) and Multiple Sclerosis

(MS) is unclear.A retrospective cohort analysis was conducted using the Mariner claims dataset. Patient records for a diagnosis of AD or MS starting 12 months post T2D diagnosis. Patients were required to be actively enrolled in Mariner claims records for six months prior and at least three years after the diagnosis of T2D without a history of previous neurodegenerative disease. Risk analysis was used to determine the association between A-HgM exposure and diagnosis of AD and MS. Risk analysis for MS was conducted in different age and sex subpopulations of T2D patients. A propensity score approach was used to minimize measured and unmeasured selection bias. The analyses were conducted between January 1st and April 28th, 2021.In T2D patients younger than 45 years old, T2D therapy exposure was associated with a reduced risk of developing MS (RR: 0.22, 95%CI: 0.17-0.29, p-value <0.001). In contrast, exposure to these drugs in patients older than 45 was associated with an increased risk of MS with women exhibiting greater risk (RR: 1.53, 95%CI: 1.39-1.69, p <0.001) than men (RR: 1.17, 95%CI: 1.01-1.37, p =0.04). Additionally, in patients older than 45 years old, A-HgM exposure was associated with a reduced risk of developing AD (RR, 0.61; 95% CI, 0.59-0.62; $p < 0.001$). Mean follow-up was 6.2 years with a standard deviation of 1.8 years.Exposure to A-HgM in patients with T2D was associated with reduced risk of MS in patients younger than 45 whereas in patients older then 45, exposure to T2D drugs were associated with an increased risk of MS, particularly in women. In older patients, A-HgM were associated with a decreased risk of AD.This study was supported by grants from the NIA/NIH grants P01AG026572 (Perimenopause in Brain Aging and Alzheimer's Disease), T32AG061897 (Translational Research in Alzheimer's Disease and Related Dementias [TRADD]), and R37AG053589 (Aging and Estrogenic Control of the Bioenergetic System in Brain) and the Women's Alzheimer's Movement to RB. R25 NS107185 (Undergraduate Readying for Burgeoning Research for American Indian Neuroscientists) and P01AG026572 (Peripheral Immune Activation on the Road to Development of Alzheimer's Disease) to KR.

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Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant P01AG026572

Title: Accelerated midlife endocrine aging in hAPOEε4/4 females

Authors: *T. WANG[1], Z. MAO[2], N. DELATORRE[1], J.-P. WIEGAND[2], R. D. BRINTON[1]; [1]Univ. of Arizona, [2]Univ. of Arizona, Tucson, AZ

Abstract: Age, female sex and *APOEε4* genotype are the greatest Alzheimer's (AD) risk factors, with a stronger *APOEε4* link to AD in women. The APOE-sex interaction is also evident in h*APOE* mouse models where h*APOEε4* induces more severe neurodegeneration and cognitive deficits in female mice. However, the mechanisms by which these two risk factors converge to disrupt brain function remain elusive.To investigate effect of *APOE* genotype on midlife endocrine aging, 6-, 9- and 15-month h*APOEε3/3* and h*APOEε4/4* female mice were stratified into 3 different endocrine aging groups based on vaginal cytology profiles: regular cyclers (consistent 4-5 day cycles), irregular cyclers (of 6-9 day cycles), and acyclic (no cycling >9 days). Plasma levels of metabolomic makers were measured. Brain mitochondrial function was quantified.Our results demonstrated that h*APOEε4/4* females exhibited accelerated endocrine aging evidenced by increased magnitude of brain metabolic defect coupled with inability to mount an adaptive bioenergetic response. Systems biology of endocrine aging initially identified in perimenopausal rat model were replicated in the h*APOEε3/3* mouse model. In contrast, h*APOEε4/4* females exhibited accumulation of adipose tissue with high plasma triglyceride and accelerated ketone body dysregulation during perimenopausal transition, indicating deficits in adaptive metabolic response required for sustaining brain metabolic demand during aging. Further, h*APOEε4/4* females exhibited greater perimenopause- and menopause-induced brain mitochondrial dysfunction.Outcomes of these analyses provide a plausible mechanistic pathway underlying the greater risk of AD in *APOEε4* females. Further, these data indicate that the bioenergetic crisis underlying metabolic reprogramming in brain are greater in the *APOEε4* female brain while compensatory adaptive responses are compromised. These findings provide a rational mechanistic precision medicine approach to intervene during midlife to prevent or delay the onset of the prodromal / preclinical stage of AD.

Disclosures: T. Wang: None. **Z. Mao:** None. **N. Delatorre:** None. **J. Wiegand:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant P01-AG026572
Women's Alzheimer Movement

Title: Anti-inflammatory drugs as risk modifiers for Alzheimer's disease and other neurodegenerative disorders: a step towards precision prevention

Authors: *H. CORTES-FLORES, R. D. BRINTON;
Univ. of Arizona, Univ. of Arizona, Tucson, AZ

Abstract: Inflammation is known to play a key role in Alzheimer's disease (AD) pathology, affecting the onset and progression of the disease and having a huge impact during the prodromal stage of the disease, which occurs 15 to 20 years prior to the onset of symptoms. It is therefore hypothesized that anti-inflammatory therapy use during mid-life can modify the risk of developing AD. This study aimed to determine if persons treated with anti-inflammatory drugs showed a reduced risk of developing AD and other neurodegenerative diseases (NDDs), and if this risk varied between women and men. We conducted a retrospective analysis using an insurance claims dataset that included 91 million people. The study included people older than 60 years, with no history of neurosurgery or NDD. The anti-inflammatory drugs included in the study were corticosteroids (CS), non-selective COX-1 and 2 inhibitors (COX1&2), and COX-2 selective inhibitors (COX2). To minimize bias, a propensity score matching was used to adjust for age, gender, cci and comorbidities. The final study population included 225,742 patients, who were surveyed for a diagnosis of AD or other NDD after at least 1 year of anti-inflammatory drug exposure. Statistical analyses were performed to compare the incidence of AD and other NDDs between the treat and control group. Anti-inflammatory use was associated with a decreased risk of NDDs (RR [95%CI]: 0.42 [0.41–0.44]; *P*<.001), AD (RR [95%CI]: 0.35 [0.33–0.37]; *P*<.001), non-AD dementia (RR [95%CI]: 0.48 [0.46–0.50]; *P*<.001), Multiple Sclerosis (MS) (RR [95%CI]: 0.28 [0.20–0.40]; *P*<.001), Parkinson's Disease (PD) (RR [95%CI]: 0.40 [0.37–0.43]; *P*<.001), and ALS (RR [95%CI]: 0.45 [0.30–0.66]; *P*<.001) compared to non-use. Sex differences analysis indicated that women exhibited greater risk reduction than men in NDDs when grouped together. Additionally, we found a similar AD risk reduction profile for each type of anti-inflammatory drug (CS: RR [95%CI]: 0.32 [0.30–0.34];*P* <.001; COX1&2: RR [95%CI]: 0.24 [0.23–0.26]; *P*<.001; COX2: RR [95%CI]: 0.21 [0.17–0.25]; *P*<.001) with no sex differences.Results reported in this study are consistent with current data indicating an association between anti-inflammatory therapies and reduced risk of AD and other aged-related NDDs. The findings support a mechanistic role of inflammation in age-associated neurodegenerative diseases and the impact of early intervention of anti-inflammatory therapeutics to reduce risk of AD, PD, MS and ALS pointing out the importance of these drugs in the development of new precision prevention strategies for AD and multiple age-related NDDs.

Disclosures: H. Cortes-Flores: None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NEUTherapeutics.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant P01AG026572 to RDB
NIH/NIA Grant 1T32AG061897 to RDB

Title: Interferon-γ Induces CNS inflammatory cascade in hAPOE mice following peripheral injection

Authors: *H. VAN ROSSUM, N. DELATORRE, A. MISHRA, A. BHATTRAI, A. RAIKES, K. RODGERS, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Dysregulation of inflammatory processes is a hallmark feature of Alzheimer's disease (AD). Previous findings from our group demonstrated upregulated immune transcripts in the humanized APOE (h*APOE)* mouse model that were related to T lymphocyte expression, microglial activation, and interferon signaling. These findings were translationally validated in the human AD brain and identified sex differences in inflammatory regulators during midlife aging. Based on this, we hypothesized that 1) increased peripheral expression of the pleiotropic cytokine interferon gamma (IFN-γ) during female midlife aging may induce CNS neuroinflammation 2) IFN-γ driven neuroinflammation is modulated by APOE genotype. With this study we test if intraperitoneal administration of peripheral IFN-γ induces genotype-dependent neuroinflammatory events in the h*APOE* ε3/3 and h*APOE* ε4/4 mouse brain. To evaluate the role of IFN-γ in female midlife aging and AD risk, female h*APOE* mice were intraperitoneally treated with recombinant IFN-γ for 9 days during midlife. Inflammatory profiles were obtained using 1) multi-color flow cytometry to assess microglial reactivity, phagocytosis, oxidative stress, and the presence of lymphocytes 2) Meso-scale Discovery ELISA assays to quantify β-Amyloid (Aβ) 40, Aβ-42, and inflammatory cytokine levels in plasma and cortex 3) targeted quantitative RT-PCR to determine immune RNA transcript levels in hippocampus. IFN-γ treated animals exhibited upregulation of markers that perpetuate inflammation including plasma cytokine expression of TNF-α ($p=0.003$), IL-2 ($p=0.0.045$), IL-10 ($p=0.001$), and IFN-γ ($p=0.010$). APOE genotype-dependent immune response was detected in brain T lymphocytes ($p=0.020$) and activated microglial phagocytosis ($p=0.040$). Plasma Aβ-40 levels were increased in hAPOEε4/4 ($p=0.013$) and a reduction in the Aβ 42:40 ratio was identified in IFN-γ treated mice ($p=0.006$) consistent with the human AD prodrome. IFN-γ treated mice exhibited a hippocampal transcriptomic profile consistent with inflammatory aging. Activation of interferon-γ signaling during midlife induced APOE genotype-dependent response in the CNS. These results support further investigation of the interaction between APOE genotype and exacerbation of neuroinflammation in female midlife aging. Precision immune therapeutic strategies that target peripheral inflammation during early midlife aging have the potential to reduce risk of AD.

Disclosures: H. Van Rossum: None. **N. Delatorre:** None. **A. Mishra:** None. **A. Bhattrai:** None. **A. Raikes:** None. **K. Rodgers:** None. **R.D. Brinton:** Other; NeuTherapeutics.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant T32AG061897
NIH/NIA Grant R01AG057931
NIH/NIA Grant RF1AG059093
NIH Grant S10OD025016

Title: Hypoactivity predicts APOE-ε4 carrier status and elevated triglyceride levels in a humanized APOE mouse model of Alzheimer's disease

Authors: *J. W. MCLEAN, A. BHATTRAI, F. VITALI, A. RAIKES, J.-P. WIEGAND, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer's Disease (AD) remains the largest contributor to neurodegenerative dementia worldwide. More than 95% of AD cases are idiopathic and influenced by several interacting risk factors. Age is the greatest risk factor for AD and the predominant genetic risk factor for AD is *APOE-ε4* carrier status. Additionally, women are nearly twice as likely to develop AD. This study investigates these risk factors using the JAX mouse model of AD with targeted replacement of murine *Apoe* with human *APOE-ε3* or *APOE-ε4*. Male and female mice (total N=76; 23.27±1.21 months) underwent open field, novel object recognition (NOR), EchoMRI body composition analysis, peripheral metabolic and amyloid biomarker assays, and a subset (n=45) received ^{18}F-FDG-PET imaging of cerebral glucose uptake. A Gaussian mixture model cluster analysis based on behavioral parameters identified two distinct clusters. Cluster 1 was characterized by further distance traveled in open field ($p=0.0016$), familiarization ($p<0.0001$), and NOR ($p<0.0001$), compared to Cluster 2. Additionally, Cluster 1 animals exhibited greater object interaction time during both familiarization ($p<0.0001$) and NOR ($p=0.0294$). Interestingly, Cluster 1 had an equal distribution of *APOE-ε4* carriers and non-carriers while Cluster 2 had a significant overrepresentation of mice with at least one *APOE-ε4* allele ($p=0.0375$). The distribution of males and females did not differ between clusters. EchoMRI-based body composition analysis indicated no weight, lean tissue percentage, or adipose tissue percentage differences between clusters. However, males had greater total weight and lean mass compared to females within each cluster (both $p<0.0001$). Further, females had lower cerebral glucose uptake than males ($p=0.0236$). There were no sex or cluster effects in fasting blood glucose, ketone bodies, or plasma amyloid-beta (Aβ) levels. However, mice in cluster 2 had elevated plasma triglyceride levels ($p=0.0384$). These results suggest that both sex and behavioral performance clusters in aged humanized *APOE* mice differ in other AD-relevant parameters, including *APOE-ε4* carrier status, cerebral glucose uptake, body composition, and triglyceride levels. Differing bioenergetic profiles may precede cognitive changes in these mice leading to the behavioral clustering observed here, mimicking early clinical presentations of AD in humans.

Disclosures: **J.W. McLean:** None. **A. Bhattrai:** None. **F. Vitali:** None. **A. Raikes:** None. **J. Wiegand:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA RF1AG057933
NIA T32 AG061892
NIA R21 AG065819

Title: Relative analysis of the Apolipoprotein E2 and E4 variants on functional connectome topology following tau seeding

Authors: *M. FEBO[1,2], M. POMPILUS[2], D. BORCHELT[1], T. WILLIAMS[1], P. CHAKRABARTY[1];
[1]Dept of Neurosci., [2]Dept of Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Allelic variants of the cholesterol carrier protein Apolipoprotein-E (APOE) are linked to varying degrees of risk to develop Alzheimer's disease (AD). Individuals with the epsilon-4 allele (APOE-ε4) are at higher risk whereas those with APOE-ε2 are protected against AD. Previous research using human APOE knockin mice bred to co-express human mutant P301S tau (PS19) demonstrated that APOE genotype alters the course of tauopathy following hippocampal K18-tau seeding (Williams *et al.,* Acta Neuropathologica Communications, 2022, 10:57). We were particularly interested in examining if there are any differences between APOE4 and APOE2 as these displayed equivalent burden of induced tauopathy and gliosis following intra-hippocampal tau seeding. Towards this, we tested whether APOE genotype ε4 vs ε2 (Tg-E4 vs Tg-E2) affects functional connectivity across densely sampled areas of the cortex, striatum, thalamus, hippocampus, amygdala, and other forebrain regions. Nontransgenic (nTg) and Tg (PS19) mice with either E4 or E2 genotype (n=4-5/group) were scanned on an 11.1 Tesla MRI under light sedation (continuous dexmedetomidine and 0.5% isoflurane) using the following parameters: single shot echo planar images with echo time of 15 ms and repetition time of 2 seconds (600 total repetitions; 0.3mm^2 in plane resolution, 0.7 mm slice thickness n 17 slices). Matrices were processed using brain connectivity toolbox to calculate global and local network metrics and average networks per group were visualized using BrainNet Viewer. In general, network graph measures reflecting global organization of functional connectivity were similar between nTg-E2,4 and Tg-E2, and these in turn differed from Tg-E4 mice. Tg-E4 mice had higher clustering coefficient, lower average path lengths, and higher efficiency than Tg-E2 and nTg-E2,4 mice. This topological pattern is indicative of high nodal integration and efficiency in Tg-E4 mice. Neuronal changes in response to tauopathy in unsampled regions of the network might underlie these functional adaptations in Tg-E4 mice. Conversely, Tg-E4 mice had lower modularity compared to nTg-E2,4 and Tg-E2 mice, a result indicative of a loss of segregation of function across groups of nodes. Our data provides initial evidence that APOE genotype significantly modifies neuronal activity and regional communication in the presence of

neuroanatomic transmission of tau initiated in the hippocampus. These changes were independent of tau burden, indicating that these functional connectivity properties are inherent and distinctive for each APOE.

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Poster

617. ApoE and Associated Pathways

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Program #/Poster #: 617.14

Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Apoe4 disrupts protein-protein signaling networks at the blood-brain barrier and synaptic interactome in mice

Authors: *B. ZLOKOVIC[1], K. KISLER[1], A. NIKOLAKOPOULOU[1], A. SAGARE[1], Y. WANG[1], G. BARISANO[1], M. HUUSKONEN[1], F. GAO[2], B. WILKINSON[1], M. COBA[1]; [1]Keck Sch. of Med. of USC, Los Angeles, CA; [2]Caltech Bioinformatics Resource Ctr., Caltech, Pasadena, CA

Abstract: Apolipoprotein E4 *(APOE4)* is the main susceptibility gene for Alzheimer's disease (AD). *APOE4* exerts cerebrovascular toxic effects resulting in blood-brain barrier (BBB) breakdown both in humans and *APOE4* transgenic mice. Moreover, BBB dysfunction predicts cognitive decline and precedes synaptic deficits in *APOE4* human carriers. It remains unknown, however, how *APOE4* affects BBB and synaptic function at a molecular level. Here, we performed a large-scale analysis of protein phosphorylation and quantitative proteomic analysis in brain capillaries isolated from APOE*4* and *APOE3* knock-in mice using a phosphopeptide-enrichment method followed by liquid chromatography-mass spectrometry (LC-MS). We then used RNAseq-guided analysis of phosphoproteome and proteome in brain endothelial cells of the BBB and BBB-associated mural cells pericytes to study molecular changes at the BBB in relation to functional changes in BBB integrity studied by DCE-MRI (dynamic contrast enhanced magnetic resonance imaging) and tissue analysis, and changes in synaptic function studied by proteome analysis. Our data suggest specific disruption of tightly connected components of the cell adhesion machinery directly connected to the dysregulated cytoskeleton

protein network, clathrin-mediated transport and translation in endothelium, and dysfunctional transcription and RNA-splicing suggestive of DNA damage in pericytes. These molecular changes were consistent with BBB damage seen by DCE-MRI analysis in the cortex and hippocampus and accumulation of peri-capillary fibrinogen deposits, and loss of pericyte seen in these regions. Post-synaptic PSD95 analysis indicated a normal protein network in young *APOE4* mice at a stage when the BBB integrity was already disrupted, that was followed at a later stage by development of a critically disrupted synaptic interactome at multiple levels (e.g., 45 proteins including glutamate receptors, the core scaffold machinery of PSD, protein kinases, etc) indicating synaptic deficits that correlated with the observed behavioral changes. Thus, *APOE4* disrupts protein-protein interactions and signaling mechanisms in endothelium and pericytes revealing molecular signature of a progressive BBB failure which we show precedes changes in synaptic interactome and function in mice.

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Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 617.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Deakin University Postgraduate Research Scholarship
NHMRC Australia (Grant 299831)
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NHMRC Australia (Fellowship 1174060)
NHMRC Australia (Fellowship RM34909)

Title: Genetic polymorphism in BIN1 rather than APOE is associated with poor recognition memory among men without dementia

Authors: *K. MEHTA[1], M. MOHEBBI[1], J. A. PASCO[1], L. J. WILLIAMS[1], K. WALDER[1], B. L. NG[2], V. GUPTA[1];
[1]Deakin Univ., Geelong, Australia; [2]Barwon Hlth., Geelong, Australia

Abstract: INTRODUCTION:Alzheimer's disease (AD) is a multifactorial disease that involves an interplay between several genetic and environmental factors. Despite this, research is mainly focused on the role of the apolipoprotein E (*APOE*) ε4 allele in the presentation of AD, as this remains the most studied genetic risk factor to date. Interestingly, AD can also develop among individuals without the *APOE* risk allele, highlighting the need to investigate other risk-conferring genetic polymorphisms.Large genome-wide association studies have identified several susceptibility loci linked to high risk of AD. Among them, the single-nucleotide

polymorphism (SNP) rs744373 in the bridging integrator 1 (*BIN1*) gene has displayed the highest effect size for AD, second only to the *APOE* ε4 allele. However, the impact of *BIN1* on multiple cognitive domains has not been explored in large population-based cohorts.
OBJECTIVES:The current study investigates the association between *BIN1* rs744373 SNP and cognitive performance across different domains among healthy ageing men free of severe cognitive impairment. It also compares these findings with *APOE*.
METHODS: A cross-sectional analysis was conducted using the 15-year follow-up data and blood samples collected from 474 male participants recruited as a part of the Geelong Osteoporosis Study. Cognitive function was evaluated using the CogState Brief Battery test which assessed cognitive performance across four domains: psychomotor function, visual attention, recognition memory and working memory. Genotyping was performed for the SNPs rs429358 (*APOE* ε4), rs7412 (*APOE* ε2) and rs744373 (*BIN1*). Multivariable linear regression models adjusted for age and *APOE* were developed to investigate the association between *BIN1* carrier status and cognitive function.
RESULTS: The study participants had a median age of 65.4 years and roughly three quarters had completed secondary education (74.8%). Individuals with the *BIN1* risk allele performed poorly on the recognition memory task as compared to those without the risk allele. The average scores were 0.03 units lower for individuals with the *BIN1* risk allele. However, this was in contrast with the *APOE* ε4 carriers who displayed better performance on the recognition task and their average scores were 0.03 units higher than non-carriers.**DISCUSSION**:
The present study demonstrates that genetic variation in *BIN1* is a better predictor of recognition memory than *APOE*. Thus, it is important to investigate the effect of genetic risk factors other than *APOE* on different cognitive domains and their biological function in the brain as this may improve our understanding of the pathophysiology of AD.

Disclosures: K. Mehta: None. **M. Mohebbi:** None. **J.A. Pasco:** None. **L.J. Williams:** None. **K. Walder:** None. **B.L. Ng:** None. **V. Gupta:** None.

Poster

617. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 617.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ZEN-20-644609

Title: Deep Learning of APOE Alzheimer Risk Gene Effects on the Brainusing a Convolutional Neural Network

Authors: *N. GOEL[1], P. RAJAGOPALAN[2], P. THOMPSON[1];
[1]USC, Los Angeles, CA; [2]Radiology, Keck Sch. of medicine, Lab. of Neuroimaging, UCLA, South Pasadena, CA

Abstract: Many landmark studies have identified a strong association between late-onset Alzheimer'sdisease (AD) and the common variants in the apolipoprotein E (APOE) gene. In general, theAPOE ε4 allele increases the risk for AD, whereas the ε2 allele has a protective role, but theeffects of these genotypes on the trajectory of neurodegeneration is still poorly understood. Here,we propose to apply deep learning methods using 3D T1-weighted (T1-w) structural brain MRIscans to detect brain biomarkers that show associations with APOE genotype. Such data drivenmethods may reveal underlying connections between genotypes and the disease. For thispurpose, we analyzed 3D T1-w brain MRI data from the Alzheimer's Disease NeuroimagingInitiative (ADNI), from 819 participants of whom 229 are controls. We partitioned the data as80% for training, 10% for validation, and 10% for testing. The possible genotypes examinedwere:ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4. To address class imbalance for various allelecombinations, we used resampling and data augmentation for particular allele combinations.First, we used a 3D CNN (convolutional neural network) trained from scratch, to classifysubjects as having at least one copy of APOE ε2 or not;in independent test data, this deep neuralnetwork achieved 78.4% average accuracy and 81.8% precision. Subjects were further classifiedas having APOE ε3/ε3 vs one or more copies of APOE ε2, with 74.3% accuracy and 81%precision. Subjects were also classified into APOE ε3/ε3 vs carrying at least one copy of APOEε4, with 75.5% accuracy and 76.2% precision. We further examined effects of age and sex onclassification accuracy. The mean age of all the scans was found to be 74.94 with std as 7.3454amongst which 5249 scans belonged to males and 4342 to females. The independence of themodel from the age was also kept in mind and subjects for Controls, MCI, and dementia werebalanced, it was found that the mean age of subjects for these groups were 75.08 yrs(std= +/-6.571;Males= 1548, Females= 1740), 74.35 yrs(std= +/- 7.69;Males= 2591, Females= 1752),and 96.01 yrs(std= +/- 7.644;Males= 1091, Females= 830) respectively. A small test set of acontrol group was also analyzed in order to check the independence of these subjects fromgenotype. It was found that the control group shows a high presence of APOE allele ε2 and verylow presence of ε4/ε4, making a distinction and model achieving mean accuracy 86.9% forAPOE ε2 versus other alleles. The subjects which showed Dementia showed the dominance ofAPOE ε4/ε4 whereas MCI subjects showed allele ε4 in dominance and suppression of ε2.

Disclosures: N. Goel: None. **P. Rajagopalan:** None. **P. Thompson:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); P.M.T received a grant from Biogen, Inc., for research unrelated to this study.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.01

Topic: C.03. Parkinson's Disease

Support: NIH grant R01NS071251
NIH grant P50NS094733

Title: The R1441C mutation compromises LRRK2 function in dopaminergic neuronal survival

Authors: L. CUI[1], ***A. SHAHAPAL**[1], J. SHEN[1,2];
[1]Brigham and Women's Hosp., Boston, MA; [2]Program in Neurosci., Harvard Med. Sch., Boston, MA

Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common genetic cause of PD, but how LRRK2 mutations cause PD is unresolved. LRRK2 is an evolutionarily conserved large protein containing multiple functional domains, including a Ras-of-complex GTPase domain and a kinase domain, where all pathogenic mutations reside. Three independent mutations (C/G/H) have been identified in the R1441 residue of the GTPase domain, suggesting its significance in LRRK2 normal function and PD pathogenesis, and the R1441C is highly penetrant. To determine the impact of the R1441C mutation on dopaminergic neurons, we generate *LRRK2* R1441C knock-in (KI) mice in the *LRRK1*-null background (*LRRK1*$^{-/-}$; *LRRK2* $^{RC/RC}$), to circumvent the compensatory effects of LRRK1. Relative to control groups, *LRRK1*$^{-/-}$; *LRRK2*$^{RC/RC}$ mice develop age-dependent dopaminergic neurodegeneration, as evidenced by marked decreases of dopaminergic neurons in the SNpc at the age of 20-25 months. In addition, *LRRK1*$^{-/-}$; *LRRK2*$^{RC/RC}$ mice show increases of apoptotic dopaminergic neurons in the SNpc at 15 months of age, prior to significant reduction of dopaminergic neurons. Furthermore, dopaminergic neurodegeneration is accompanied with microgliosis and accumulation of autophagic vacuoles in the SNpc of *LRRK1*$^{-/-}$; *LRRK2*$^{RC/R}$ mice. Thus, relative to wild-type LRRK2, the R1441C mutation compromises LRRK2 function in support of dopaminergic neuronal survival during aging.

Disclosures: L. Cui: None. **A. Shahapal:** None. **J. Shen:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.02

Topic: C.03. Parkinson's Disease

Support: NIH/ NIA grant R01AG072520
NIH/NINDS grant P20NS123220

Title: Characterization of Parkinson's disease mutant LRRK2-G2019S in dysregulating microglial secretion of lysosomal proteins

Authors: *B. HUANG[1], I. CHOI[2];
[1]Icahn Sch. Of Med. at Mount Sinai, New York, NY; [2]Neurol., Ichan Sch. of Med. At Mount Sinai, New York, NY

Abstract: Characterization of Parkinson's disease mutant LRRK2-G2019S in dysregulating microglial secretion of lysosomal proteins
Bik Tzu Huang, Ravi Ghotra, Insup Choi and Zhenyu Yue
Background: G2019S LRRK2, one of the mutant forms of LRRK2 exhibiting increased kinase activity, has been found in both familial and sporadic Parkinson's disease (PD). Accumulating evidence has shown that a subset of Rab GTPases, regulators for intracellular vesicle trafficking, become phosphorylated by LRRK2. We recently found that Rab12 is a physiological substrate of LRRK2 in the mouse brain. In our scRNAseq analysis, we found LRRK2 is highly expressed in microglia of human PD brains. Therefore, we sought to investigate whether and how LRRK2 kinase activity affects Rab12 phosphorylation and subcellular localization in microglia.Method: We employed primary microglia cultured from G2019S-LRRK2, LRRK2 knock-out (KO) microglia and zymosan, a well-known stimulator for immune cells. After zymosan treatment, we measured the levels of phosphorylation of Rab12 by Western blot. Further, we examined the subcellular localization of LRRK2 and Rab12, in primary microglia or LRRK2 or Rab12-transfected BV2, a microglial cell line, by immunostaining and biochemical subcellular fractionation. Lastly, we did media collection to observe secretion differences between genotypes. Results: We observed that pRab12 is found primarily in membrane compartments in a subcellular fractionation assay. G2019S-LRRK2 and Rab12 are highly colocalized at Lamp1-positive late endosome/lysosome structures. Also, G2019S-LRRK2 showed higher secretion of lysosomal proteases, Cathepsin D and B in response to zymosan.Conclusions: Rab12 is a LRRK2 substrate in cultured primary microglia and LRRK2 interacts with Rab12 in the lysosome. Our data suggests that PD mutant LRRK2-G2019S alters microglial secretion of lysosomal proteins.

Disclosures: B. Huang: None. **I. Choi:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.03

Topic: C.03. Parkinson's Disease

Title: Advancing drug discovery for Parkinson's disease through the development of high throughput assays using industrialized human iPSC derived cell models

Authors: S. SCHACHTELE, A. FATHI, J. LIU, R. FIENE, S. DICKERSON, C. CARLSON, *E. JONES, S. HILCOVE;
FUJIFILM CDI, Madison, WI

Abstract: Induced pluripotent stem cell (iPSC) technology has opened the possibility of taking somatic cells from virtually any human donor and converting them into virtually any cell type imaginable. With the help of the Parkinson's Progression Markers Initiative (PPMI), as part of The Michael J. Fox Foundation (MJFF), we generated iPSC lines from patients with Parkinson's

disease (PD) carrying known risk-associated gene mutations and clinical data supporting symptoms of PD. Furthermore, we have performed large-scale differentiations of these iPSC lines into biologically relevant midbrain dopaminergic neurons (i.e., iCell® DopaNeurons) to facilitate PD-focused assay development and drug screening. The etiology of dopaminergic neuron cell death in PD is complex, involving multiple factors that include mitochondrial dysfunction, impaired endosomal/lysosomal protein degradation, alpha-synuclein and tau aggregation, and neuroinflammation. Here, we utilized dopaminergic neurons from PD donor-derived iPSCs harboring either the LRRK2 G2019S or GBA N370S mutation and compared them to apparently healthy normal (AHN) iCell DopaNeurons. All cells showed similar marker expression and neuronal purity characteristic of dopaminergic neurons. We then seeded these cells into multiple assay formats, specifically investigating neuronal activity (multielectrode array), neurite outgrowth and degeneration, cell death, metabolism (Seahorse), alpha-synuclein accumulation, and GCase activity. For high-throughput screening, we developed a GCase activity assay and confirmed that known GBA-modulating compounds, such as cyclodextrin, modify the reduced GCase activity observed in iCell DopaNeurons containing either LRRK2 G2019S or GBA N370S mutations. This study shows the utility of PD-donor derived dopaminergic neurons within multiple disease-relevant assays, suggesting their potential use in drug screening and therapeutic validation.

Disclosures: **S. Schachtele:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **A. Fathi:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **J. Liu:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **R. Fiene:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **S. Dickerson:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **C. Carlson:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **E. Jones:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **S. Hilcove:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc..

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.04

Topic: C.03. Parkinson’s Disease

Support: NIH RO1 NS102257
NS 117465
PF-FBS 1931

Title: Cellular and subcellular localization of Rab10 and phospho-T73 Rab10 in the Brain

Authors: ***V. SINGH**[1], M. MENARD[1], D. THRASHER[1], G. SERRANO[2], T. BEACH[2], H. ZHAO[3], L. VOLPICELLI-DALEY[1];

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Abstract: Cellular and subcellular localization of Rab10 and phospho-T73 Rab10 in the Brain

Authors: Vijay Singh[1], Marissa A. Menard[1], Drake R. Thrasher[1], Geidy E. Serrano[2], Thomas G Beach[2], Hien T. Zhao[3], Laura A. Volpicelli-Daley Ph.D[1]
Affiliations: [1]Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham, Birmingham, AL 35294, USA. [2]Department of Neuropathology, Banner Sun Health Research Institute, Sun City, AZ 85351, USA [3]Ionis Pharmaceuticals Inc, Carlsbad, CA 92010, USA
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AbstractParkinson's disease (PD) is the most common neurodegenerative motor disease characterized by Lewy bodies and loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). Autosomal dominant pathogenic mutations in Leucine-rich repeat kinase 2 (LRRK2) cause Parkinson's disease (PD).The most common G2019S-LRRK2 mutation increases the kinase activity of LRRK2 which, in turn, hyper-phosphorylates its substrates. One of these substrates includes Rab10 which is phosphorylated at a conserved Thr73 (pRab10), and is one of the most abundant LRRK2 Rab GTPases expressed in various tissues. Moreover, Rab10 is involved in both PD, and Alzheimer's disease. Because of the role of Rab10 in neurodegenerative disease, pinpointing the cellular and subcellular localization of Rab10 and pRab10 in the brain can help understand its functional role, and how abnormal post-translational modifications could impact function. Our results determine the localization of Rab10 and pRab10 in glia, neurons, and subcellular organelles. Rab10 and pRab10 were expressed in the cortex, striatum, and the substantia nigra pars compacta. While Rab10 colocalized with endoplasmic reticulum, lysosome and trans-Golgi network markers, pRab10 did not localize to these organelles. pRab10,
however, did overlap with markers of the presynaptic terminal in both mouse and human cortex.

Disclosures: V. Singh: None. **M. Menard:** None. **D. Thrasher:** None. **G. Serrano:** None. **T. Beach:** None. **H. Zhao:** None. **L. Volpicelli-Daley:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.05

Topic: C.03. Parkinson's Disease

Support: NIH grant NS110188
Hough Family Foundation

Title: The Impact of LRRK2 on Cellular Tau Pathology of Parkinson's Disease in iPSC Derived Neurons

Authors: *L. RILEY-DIPAOLO[1], A. MAMAIS[1], M. J. LAVOIE[2];
[1]Neurol., [2]Ctr. for Translational Res. in Neurodegenerative Disease, Fixel Inst., Univ. of Florida, Gainesville, FL

Abstract: Tau, a microtubule-binding protein that contributes to their stabilization, abounds within the CNS. Hyperphosphorylation of Tau is linked to neurofibrillary tangle (NFT) formation, a pathological hallmark of Alzheimer's Disease and other tauopathies. Tau phosphorylation can disrupt its binding to microtubules and is believed to drive protein aggregation. Parkinson's Disease (PD) features a progressive loss of dopaminergic neurons in the substantia nigra leading to loss of motor function. Autosomal dominant missense mutations in LRRK2 are the most common genetic cause of PD. While idiopathic PD is characterized by the presence of Lewy body inclusions comprised of alpha-synuclein protein, most LRRK2-PD cases present with PSP-like NFTs, as opposed to classic Lewy pathology. Here, we seek to examine the relationship between LRRK2 mutation and Tau biology in human neurons. The aims of this project are to: a) establish a model system of Tau pathology in iPSC-derived human neurons, and b) investigate the effect of PD-linked LRRK2 mutations on Tau neuropathology and its biochemical characteristics including metabolism, phosphorylation, and turnover rates.We developed a neuronal model for studying Tau kinetics and toxicity utilizing vectors expressing WT and a 2x Tau mutant (P301L and S320F). Tau variants were fused to the photoconvertible Dendra2 tag enabling identification of Tau subcellular localization and its morphology, as well as turnover using live cell imaging. Other model systems demonstrate that the synthetic 2x Tau variant reliably induces spontaneous aggregation. Preliminary data suggest that following transient transfection at DIV 7, 2x Tau is sequestered into puncta along neurites at a higher rate than WT Tau, noted as early as DIV 16. Furthermore, we observed aggregated Tau protein in the soma that draws parallels to documented pathology in humans. The morphological readouts we are assessing include Tau aggregation properties and its effect on neurite retraction.Utilizing the Dendra2 tag, we developed a photoconversion protocol in induced neurons to examine Tau turnover and clearance rates. Dendra2 constructs were transiently transfected and expressed for as few as 3 days prior to photoconversion. Neurons were photoconverted from green to red emission with exposure to blue light, then imaged using automated microscopy. High content imaging will allow for analyses of protein signal intensity, neurite morphology, and Tau clearance/synthesis rates. Using this optimized system following this preliminary data, we will extend this work to examine whether LRRK2 G2019S or R1441C mutations affect the cellular pathology of 2x Tau.

Disclosures: L. Riley-DiPaolo: None. **A. Mamais:** None. **M.J. LaVoie:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.06

Topic: C.03. Parkinson's Disease

Support: NIH RO1 NS102257
NIH RO1 NS 117465

Title: Lrrk2-g2019s regulation of darpp32 influences glutamatergic receptor expression

Authors: *A. KAMATH, L. A. VOLPICELLI-DALEY, V. SINGH;
Heersink Sch. of Medicine, Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Parkinson's Disease (PD) is a neurodegenerative disease characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain. Over 10 million people worldwide are currently diagnosed with PD, and about 15% of PD cases are familial and majority of these are attributed to mutations in the leucine rich-repeat kinase 2 (LRRK2) gene. LRRK2 is highly expressed in medium spiny projection neurons (SPNs) of the striatum. SPNs express either D1 or D2 dopamine receptors that relay information to the basal ganglia and thalamic nuclei about motor decisions that are then sent to the cortex. The LRRK2-G2019S mutation influences SPN excitability by inhibiting activity of protein kinase A (PKA) and regulates its ability to phosphorylate dopamine and cAMP-regulated phospho-protein Mr 32,000 (DARPP32). Selective phosphorylation of DARPP32 regulates protein phosphatase-1 (PP-1) activity and may play a key role in regulating activation of AMPA and NMDA receptors, both of which affect long term potentiation (LTP). Revealing a connection between the LRRK2-G2019S mutation and glutamate receptor activity via the phosphorylation of DARPP32 will be groundbreaking in allowing researchers to explore the mechanisms by which the basal ganglia pathways and PD dopaminergic signaling pathway is affected by the LRRK2-G2019S mutation[SV(1] . Preliminary results indicate a consistent reduction of DARPP32 in the cortex of LRRK2-G2019S knock-in mice, and I am further investigating the effect of the mutation on glutamate receptors in primary mouse models.

Disclosures: **A. Kamath:** None. **L.A. Volpicelli-Daley:** None. **V. Singh:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.07

Topic: C.03. Parkinson's Disease

Support: American Parkinson Disease Association (Postdoctoral fellowship)
Parkinson's foundation (Postdoctoral fellowship PF-FBS-2025)
National Institute of Health (NIH/NINDS R01 NS105432)
Van Andel Institute

Title: LRRK2 activation and the endolysosomal system in a parkinson's-linked D620N VPS35 rodent model.

Authors: *D. SARGENT, A. OTERO, V. HOWLAND, D. J. MOORE;
Dept. of Neurodegenerative Sci., Van Andel Inst., Grand Rapids, MI

Abstract: Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) and *vacuolar protein sorting ortholog 35* (*VPS35*) genes cause late-onset, autosomal dominant familial Parkinson's disease (PD). It is widely accepted that LRRK2 mutations enhance LRRK2 kinase activity, which induces multiple cellular defects including an impairment of the endolysosomal pathway that degrades and recycle protein aggregates and damaged organelles. Interestingly, the PD-linked *D620N VPS35* mutation has been reported to increase LRRK2 substrate phosphorylation in both a *D620N VPS35* knockin (KI) mouse model of PD and in PBMCs and monocytes derived from PD subjects bearing a *D620N* mutation. However, it remains unknown whether LRRK2 activation contributes to PD in these *VPS35*-linked subjects and by which mechanisms. Here, we explore LRRK2 kinase activation in primary cortical cultures derived from *D620N VPS35* KI mice and evaluate its potential impact on the endolysosomal pathway. We first demonstrate that LRRK2 substrate phosphorylation (i.e. Rab8a, Rab10 and Rab12) is markedly increased in distinct brain regions and peripheral tissues from heterozygous and homozygous *D620N VPS35* KI mice, and this increase is abolished by crossing these mice onto a *LRRK2* null background. In the brain of *D620N VPS35* KI mice, we find that Rab12 is robustly phosphorylated relative to Rab10, but we fail to observe a corresponding increase in LRRK2 autophosphorylation at Ser1292. These data suggest that D620N VPS35 enhances LRRK2 substrate phosphorylation rather than LRRK2 kinase activity in general. We next used primary cultures derived from *D620N VPS35* KI mice to understand which brain cells exhibit LRRK2 activation since current phospho-Rab antibodies do not work well for immunohistochemistry on brain sections. Our data suggests that LRRK2 kinase hyperactivation is prominent in cortical astrocytes but not cortical neurons where it localizes throughout the cell, including upon lysosomes. LRRK2 is activated and partially recruited to lysosomes in *D620N VPS35* KI astrocytes, which is reduced by LRRK2 kinase inhibition. The basal levels of endolysosomal pathway markers, autophagic flux or lysosomal protease activity are not significantly altered in *D620N VPS35* KI astrocytes, suggesting that the impact of LRRK2 hyperactivation on lysosomal function is limited. Interestingly, *VPS35* KI astrocytes showed reduced lysosomal enlargement compared to wild-type cells upon treatment with lysosomal stressors. Altogether, our study suggests that LRRK2 is activated and recruited to lysosomes in astrocytes in *D620N VPS35* KI mice where it plays a role in lysosomal homeostasis upon stress.

Disclosures: D. Sargent: None. **A. Otero:** None. **V. Howland:** None. **D.J. Moore:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.08

Topic: C.03. Parkinson's Disease

Title: Characterization of a novel small-molecule LRRK2 inhibitor WI-211094 as a potential therapeutic agent for Parkinson's disease

Authors: *J. SEO[1], J. LEE[1], B. YOU[1], J. HEO[1], S. HAN[1], K. PARK[1], H. SHIN[1], D. HO[2], H. CHOI[3], G. LEE[4], S. KANG[1];
[1]Whan In Pharmaceut. Co., Ltd., Seoul, Korea, Republic of; [2]InAm Neurosci. Res. Center, Sanbon Med. Center, Col. of Medicine, Wonkwang Univ., Gunpo, Korea, Republic of; [3]Col. of Pharm. and Inst. of Pharmaceut. Sciences, CHA Univ., Pocheon, Korea, Republic of; [4]Office of Small & Medium Enterprises Support, Korea Res. Inst. of Chem. Technol., Daejeon, Korea, Republic of

Abstract: Inhibition of leucine-rich repeat kinase 2 (LRRK2) is a potential target for disease-modifying therapy in Parkinson's disease (PD) since mutations in its kinase domain are known to cause familial and idiopathic PD (iPD). Here, we report WI-211094, a novel, highly potent (IC50=26 nM for WT and 6.4 nM for G2019S mutant), selective, and central nervous system (CNS)-penetrant small-molecule LRRK2 inhibitor. In an lipopolysaccharide (LPS)-treated BV2 cell model, inhibition of LRRK2 by WI-211094 decreased both reactive oxygen species (ROS) and TNF-alpha levels. Moreover, WI-211094 improved the mitochondrial dysfunction, implying its anti-neuroinflammatory effects. WI-211094 possesses high oral bioavailability in rodents and beagle dogs, and it exhibited in vivo efficacy in a PD animal model, the rotenone-induced mouse model. WI-211094 treatment rescued the expression level of tyrosine hydroxylase (TH) impaired by rotenone and reduced the phosphorylation level of alpha-synuclein in the substantia nigra (SN), indicating that WI-211094 could attenuate the toxicity of alpha-synuclein by interrupting its aggregation. In mice, WI-211094 was well tolerated up to 1000 mg/kg once daily for 2 weeks without histopathological alterations in tissues, especially lung adverse effects. Taken together, we suggest that WI-211094, a novel, advanced, and generally safe LRRK2 inhibitor, might be a potential therapeutic candidate for both iPD patients and familial PD patients with LRRK2 mutation.

Disclosures: **J. Seo:** None. **J. Lee:** None. **B. You:** None. **J. Heo:** None. **S. Han:** None. **K. Park:** None. **H. Shin:** None. **D. Ho:** None. **H. Choi:** None. **G. Lee:** None. **S. Kang:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.09

Topic: C.03. Parkinson’s Disease

Support: RSF grant 22-25-00501

Title: Alteration of lysosomal activities and lysosphyngolipid level in the pathogenesis of Parkinson’s disease associated with mutations in the LRRK2 gene

Authors: *T. USENKO[1,2], K. BASHAROVA[1], A. BEZRUKOVA[1], G. BAYDAKOVA[3], A. KOPYTOVA[1], M. BELETSKAYA[2], A. EMELYANOV[1,2], I. MILIUKHINA[1,2,4], A. TIMOFEEVA[2], E. ZAKHAROVA[3], S. PCHELINA[1,2];
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Abstract: Parkinson's disease (PD) is a common neurodegenerative disorder. G2019S mutation in leucine-rich repeat kinase 2 (LRRK2) is common genetic risk factor for PD. However, molecular mechanisms of PD associated with mutation in the LRRK2 gene remain unknown. Earlier, it was shown that the most common mutation in the LRRK2 gene, G2019S, was associated with activity of glucocerebrosidase (GCase) enzyme, encoded by the GBA gene, mutations in which are also common genetic risk factors for PD. The aim of the current work was to evaluate whether the alteration of lysosomal activities may play role in PD associated with mutation G2019S in the LRRK2 gene (LRRK2-G2019S-PD). Blood samples of 6 patients with LRRK2-G2019S-PD, 180 sPD patients and 168 controls were generated. Also, one patient with PD with double mutation, G2019S in the LRRK2 gene and N370S in the GBA gene, in heterozygous states was enrolled. The enzymatic activities of GCase and also alpha-L-Iduronidase (IDUA), galactosylceramidase (GALC), alpha-galactosidase (GLA), acid sphingomyelinase (ASMase) and their substrates concentrations (hexosylsphingosine (HexSph), globotriaosylsphingosine (LysoGb3), lysosphingomyelin (LysoSM)) were measured by liquid chromatography tandem-mass spectrometry in blood. All measurements were taken twice. No differences in enzyme activity of GCase, IDUA, GALC in LRRK2-G2019S-PD patients with sPD and controls were found ($p>0.05$). However, GALC activity was elevated in sPD compared to controls ($p=0.009$). Surprisingly, ASMase activity was decreased and GAA activity in LRRK2-G2019S-PD compared to sPD patients ($p=0.010$, $p=0.041$, respectively). While HexSph concentration was increased in LRRK2-G2019S-PD patients compared to sPD patients ($p=0.036$) and LysoGb3 concentration was increased in LRRK2-G2019S-PD patients than in sPD patients and controls ($p=0.049$, $p=0.034$, respectively). Interesting, that N370S/G2019S PD patients is characterized as expected by decreased of GCase activity and increased HexSph and also increase of GLA, GALC activities and increase of LysoSM concentration were found. Our results first demonstrated alteration of lysosomal enzyme activity and lysosphingolipid concentrations with decrease of ASMase activity and increase of HexSph and LysoGb3 concentrations in patients with LRRK2-G2019S-PD These alterations may play role in LRRK2-PD pathogenesis. **This study supported by RSF grant 22-25-00501.**

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Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.10

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation (MJFF)
NIH/NINDS R01 NS091719
NIH/NINDS R01 NS120489
Van Andel Institute

Title: Parkinson'S disease risk variant, g2385r lrrk2, enhances rab phosphorylation, neurotoxicity and neurodegeneration

Authors: *A. TRAN NGUYEN[1], R. MOSER[2], L. CUNNINGHAM[1], N. BRYANT[3], A. B. WEST[3], S. M. YOUNG, Jr.[4], D. J. MOORE[1];
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Abstract: Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene are the most common known cause of late-onset, autosomal dominant Parkinson's disease (PD). Genome-wide association studies (GWAS) consistently indicate that common variants in the *LRRK2* gene are associated with an increased risk of sporadic PD. LRRK2 is a large, multi-domain protein containing two distinct and functional enzymatic domains: a Roc-COR GTPase domain and a kinase domain. LRRK2 pathogenic mutations cluster within the central kinase (G2019S, I2020T), GTPase (N1437H, R1441C/G/H) and COR (Y1699C) domains highlighting the critical role of LRRK2 enzymatic activities in PD pathogenesis. In addition to PD-linked familial mutations, several *LRRK2* coding variants are associated with altered risk of sporadic PD in certain ethnic populations. The mechanism by which these coding variants modulate PD risk is not yet clear. In this study, we first sought to determine whether *LRRK2* common coding risk variants alter the biochemical properties of LRRK2 and mediate neurotoxicity in cell culture. We find that the PD risk variant, G2385R, reduces LRRK2 protein steady-state levels and LRRK2 cellular phosphorylation (pSer1292, pSer910 and pSer935) in cell lines. Moreover, PD risk variants (A419V, R1628P, M1646T and G2385R) commonly increase LRRK2-mediated Rab10 phosphorylation (pThr73-Rab10) compared to wild-type LRRK2. In contrast, PD protective variants (N551K and N551K-R1398H, but not R1398H alone) oppositely exhibit reduced pT73-Rab10 levels compared to wild-type LRRK2. Further studies reveal that G2385R LRRK2 expression markedly inhibits neurite outgrowth in primary cortical neurons compared to wild-type LRRK2. However, combining coding PD risk variants with a familial *LRRK2* mutation (G2019S) has a limited impact on the biochemical properties of G2019S LRRK2 or its effects on neurite outgrowth inhibition in cortical neurons. We next developed a new LRRK2 preclinical model of PD by employing recently generated helper-dependent adenovirus serotype 5 (HdAd5) vectors. We find that the striatal delivery of HdAd5 vectors expressing human G2385R LRRK2 in adult rats robustly induces the degeneration of nigral dopaminergic neurons over 6 weeks, comparable to the effects of G2019S LRRK2. The mechanism underlying the pathogenic effects of G2385R LRRK2 in the brain remains to be explored. Collectively, our study supports increased pT73-Rab10 levels as a relevant indicator of PD risk mediated by common LRRK2 coding variants and demonstrates for the first time that the PD risk variant, G2385R LRRK2, is capable of inducing PD-like neurodegenerative phenotypes *in vivo*.

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Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.11

Topic: C.03. Parkinson's Disease

Support: NS117137

Title: Delineating Neurodegenerative Interactions of VPS35 and LRRK2 in Rodent Models of Parkinson's Disease

Authors: *X. CHEN, L. CUNNINGHAM, N. LEVINE, A. TRAN NGUYEN, D. MOORE; Dept. of Neurodegenerative Sci., Van Andel Res. Inst., Grand Rapids, MI

Abstract: Mutations in the *VPS35* gene encoding a core component of the retromer complex, which is important for endosomal protein sorting, have emerged as a cause of late-onset, autosomal dominant Parkinson's disease (PD). At present, the mechanism(s) by which familial *VPS35* mutations precipitate neurodegeneration in PD are poorly understood and the extent to which mutant VPS35 interacts with other PD-linked gene products in rodent models is uncertain. VPS35 and the retromer have so far been functionally connected to the PD-linked proteins, α-synuclein, parkin, and LRRK2. Mutations in the *LRRK2* gene are the most common cause of autosomal dominant PD and have been shown to increase LRRK2 kinase activity including autophosphorylation and phosphorylation of Rab substrates. Emerging evidence suggests that *VPS35* mutations may operate upstream or in parallel to LRRK2 where they either directly or indirectly induce kinase activation that potentially mediates neurodegeneration. However, the consequences of increased LRRK2 kinase activity in *D620N VPS35* rodent models have not been formally evaluated. We previously developed a PD animal model based upon the AAV-mediated overexpression of human D620N VPS35 in the nigrostriatal pathway of adult rats that induces dopaminergic neurodegeneration and axonal damage. Our ongoing studies seek to evaluate the impact of VPS35 overexpression on LRRK2 kinase activity in commonly utilized rat genetic backgrounds. Furthermore, we are utilizing *LRRK2*-deficient models to define the contribution of LRRK2 to mechanisms associated with mutant VPS35-induced neurotoxicity. To explore the pathological significance of LRRK2 kinase activation, we assessed the impact of *LRRK2* deletion in mice on the pronounced dopaminergic neuronal loss induced by the AAV2/6-mediated overexpression of D620N VPS35 in the substantia nigra. Surprisingly, we find that *LRRK2* deletion fails to provide neuroprotection against mutant VPS35, and potentially worsens neurodegeneration, suggesting that LRRK2 does not operate downstream to mediate the pathogenic effects of mutant VPS35. Collectively, our studies are validating a potentially important common pathogenic pathway in PD and will determine whether LRRK2 kinase activity could be targeted in *VPS35*-linked PD.

Disclosures: **X. Chen:** None. **L. Cunningham:** None. **N. Levine:** None. **A. Tran Nguyen:** None. **D. Moore:** None.

Poster

618. LRRK2 Mechanisms, Targets, and Pathways

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 618.12

Topic: C.03. Parkinson's Disease

Support: DBT, Government of India; contract grant No. BT/PR21526/ MED/122/49/2016

Title: Impairment of glutamate uptake and metabolism and Nrf2-mediated glutathione machinery in astrocytes derived from LRRK2 I1371V Parkinson's disease patient iPSCs

Authors: *I. DATTA[1], R. BANERJEE[1], A. RAJ[1], R. YADAV[2], P. PAL[2];
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Abstract: With greater understanding of significance of phosphorylated α-synuclein in Parkinson's disease (PD) pathogenesis, focus is on kinase mutations, of which LRRK2 is most significant. Being a large multidomain protein, LRRK2 has several confirmed pathological mutant-variants, with their incidence showing ethnicity-biases & drug response differences. LRRK2 is expressed in dopaminergic neurons and astrocytes. Healthy astrocytes are essential for maintenance of adjacent neurons, & autopsy data from PD patients show presence of dystrophic reactive astrocytes. The LRRK2 variant I1371V, with a mutation in GTPase-domain of the enzyme, has been reported in East-Asian populations but there are no studies reported on astrocytes differentiated from this variant. Here, we aim to investigate impact of I1371V mutation of LRRK2 on astrocyte yield & biology involving glutamate uptake, glutamate metabolism, & Nrf2-mediated glutathione machinery. Astrocytes are derived from iPSCs generated from LRRK2 I1371V PD patients & age & gender-matched healthy control (HC). Yield of GFAP immunopositive population of astrocytes was similar between PD & HC differentiated cells. However, glutamate uptake was significantly reduced in the LRRK2 I1371V PD astrocytes along with lower gene expression of glutamate transporters *SLC1A2, SLC1A3*. In addition, conversion of glutamate to glutamine or alpha-ketoglutarate that enters the TCA cycle was impaired with significantly lower ATP generation in the PD iPSC-derived astrocytes. *De novo* synthesis of glutamine is impaired, causing transport of glutamine to the neurons to be also reduced, indicating disorder in the intracellular glutamate metabolism. Oxidative stress-quenching capability of the astrocytes in terms of glutathione content was significantly reduced in PD astrocytes. Gene expression of enzymes involved in glutathione machinery viz. *Glutathione Synthetase*, *Glutathione Peroxidase*, *Glutathione Reductase* & *Glutamyl Cysteine Ligase*, are also lower compared to HC astrocytes. Thus, the antioxidation property of the astrocytes is compromised due to impaired glutathione toolkit in LRRK2 I1371V PD astrocytes. Our data demonstrate conclusively that mutation in the I1371V allele of LRRK2 shows astrocytic dysfunction with respect to Nrf2 mediated anti-oxidant mechanism, ATP generation &

glutamate-metabolic profile even with comparable astrocyte yields, making the niche cells compromised in their neuro-supportive functions. It emphasizes that LRRK2 I1371V mutation is indeed pathogenic, & that these LRRK2 I1371V PD iPSC-derived astrocytes can serve as a platform to replicate patient brain astrocyte pathology in PD.

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Poster

618. LRRK2 Mechanisms, Targets, and Pathways

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Program #/Poster #: 618.13

Topic: C.03. Parkinson's Disease

Support: NIH Grant 5R00ES029986-04

Title: Lrrk2 kinase inhibition protects dopaminergic neurons from parkinson's disease-associated environmental toxicants

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Abstract: Environmental toxicants that induce mitochondrial dysfunction are associated with increased risk of Parkinson's disease (PD) and include pesticides - rotenone (ROT) and paraquat (PQ), and organic solvents - trichloroethylene (TCE) and tetrachloroethylene (PERC). Recently, we reported that protein kinase LRRK2, the most commonly inherited mutation in familial PD, exhibits aberrant kinase activity in wildtype rats exposed to environmental toxicants like TCE. Toxicant-induced LRRK2 kinase activity elevation shows neuronal pathology mirroring inherited mutations in LRRK2, such as vesicular trafficking impairment, and oxidative damage. Based on these data, we hypothesized that inhibition of LRRK2 kinase activity may attenuate toxicant-induced damage to dopaminergic (DA) neurons, and protect against neurodegeneration in idiopathic PD.
To investigate this, we first assessed the efficacy of LRRK2 kinase inhibition against environmental PD toxicants using the dopaminergic N27-A cell line exposed to 500 nM ROT, 100 µM PQ, 500 µM TCE or PERC. Toxicant exposure significantly increased reactive oxygen species (ROS) quantified using fluorescent indicator dye dihydroethidium (DHE; $p<0.001$), and was potently reduced by co-treatment with a LRRK2 kinase inhibitor (MLi2; $p >0.05$). We also observed that CRISPR-edited 293 HEK cells with the common G2019S LRRK2 mutation displayed elevated ROS at baseline, showing exacerbated phenotype with toxicant exposure compared to wildtype ($p < 0.0001$). In contrast, LRRK2 knockout cells were robustly protected from toxicant-induced ROS production ($p <0.0001$). We postulated that mitophagy may be a convergence point for LRRK2 and environmental toxicants, and may serve as a potential

mediator of its effects. We therefore assessed LC3B with TOM20 (autophagosomal ; mitochondrial markers respectively) as a mitophagy proxy in N27-A cells treated with the four toxicants mentioned prior, alone or with MLi2. Toxicant exposure significantly reduced mitophagy, and was ameliorated by MLi2 co-treatment ($p < 0.001$). We confirmed these results *in vivo. A*dult rats exposed to 6 weeks of 200 mg/kg TCE orally displayed elevated oxidative damage within DA neurons (3-nitrotyrosine, 4-hydroxynonenal), and post-lesion 3 week oral 10 mg/kg MLi2 significantly rescued this phenotype ($p < 0.0001$). Collectively, these data indicate that wildtype LRRK2 kinase activity plays a role in PD-linked environmental contaminant toxicity, which may be exacerbated in individuals with LRRK2 mutations. Pharmacological LRRK2 kinase inhibition may be protective against DA neurotoxicity by restoring mitophagy and reducing oxidative stress.

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Poster

619. Parkinson's Disease: Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 619.01

Topic: C.03. Parkinson’s Disease

Support: Fondation Maladies Rares (Project Grant No. 10877)
Fondation de France (Project Grant No. 00066525)
France Parkinson (Project Grant No. OPE FP 2020-0616)

Title: Phenotypic characterization of an ATP13A2 knockout rat model of Parkinson's disease

Authors: J. SIKORA[1,2], M.-L. AROTCARENA[1], M. DELCOURT[3], E. BALADO[3], E. BEZARD[1], P.-O. FERNAGUT[3], ***B. DEHAY**[1];
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Abstract: Parkinson’s disease (PD) is a complex, progressive neurodegenerative disease characterized by the loss of nigrostriatal dopaminergic innervation and the presence of intraneuronal inclusions called Lewy Bodies. Most PD cases are sporadic, but 10-15% of patients have a familial type. The *ATP13A2* gene encodes a transmembrane lysosomal P5-type ATPase recently identified as a lysosomal polyamine exporter. Mutations in the *ATP13A2* gene were identified as the cause of Kufor-Rakeb syndrome, a juvenile-onset form of PD. The development of a relevant and predictable PD model is still an unmet need for the research community to better understand the mechanisms underlying the pathology and to identify and validate therapeutic strategies. This project aimed to characterize the first-ever transgenic ATP13A2 knockout rat model to elucidate the underlying mechanisms of ATP13A2-associated pathology. To this end, we performed a comprehensive longitudinal characterization of

symptoms and associated neuropathology in this animal model of PD, offering new insights into PD pathogenesis and a potential tool for testing and validating therapeutic approaches.To assess whether deletion of the *ATP13A2* gene in rats can replicate human pathology, we followed developmental milestones as well as longitudinal motor assessment to evaluate akinesia and bradykinesia using the “stepping” test every 3 months up to 12 months of age and also assessed fine motor skills using a reaching task as these deficits are one of the first motor symptoms occurred in PD patients. We also evaluated whether a viral-mediated overexpression of α-synuclein exacerbates motor and cellular deficits in ATP13A2 KO rats. The behavioral assessment demonstrated specific developmental deficits in animals with a reduced and fully deleted expression of ATP13A2. ATP13A2 KO rats displayed age-dependent fine motor skills deficits and impaired locomotor habituation similar to those observed in parkinsonian patients at the early stage of motor symptom onset. We detected significant differences in astroglial activation in the substantia nigra of ATP13A2 KO, consistent with the pathology seen in PD brain patients. Overexpression of mutated human alpha-synuclein in substantia nigra showed a significant increase in protein expression without a significant effect of genotype. Additional analyses are ongoing to understand the contribution of lysosomal ATP13A2 protein in the pathogenesis of PD to further uncover which mechanisms can contribute to the occurrence of motor symptoms observed in the APT13A2 KO rats that might open new therapeutic opportunities for slowing down the degenerative process in patients with PD.

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Poster

619. Parkinson's Disease: Animal Models

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Program #/Poster #: 619.02

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation (Project Grant No. 2013-8499)

Title: Cortical Lewy Body injections induce cerebral pathological features in non-human primates

Authors: *M. LANDRY[1], M. TEIL[1], S. DOVERO[1], M. BOURDENX[1], M.-L. AROTCARENA[1], S. CAMUS[1], G. PORRAS[1], M.-L. THIOLAT[1], I. TRIGO DAMAS[2], C. ESTRADA[3], N. GARCIA CARILLO[3], M. HERRERO[3], M. VILA[4], J. OBESO[2], E. BEZARD[1], B. DEHAY[1];
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Abstract: Aggregation of α-synuclein has been implicated in several neurodegenerative diseases, termed synucleinopathies, which include Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB), and Multiple System Atrophy (MSA). These synucleinopathies are characterized by the deposit of α-synuclein aggregated in intracellular inclusions in neurons and/or glial cells. In PD and DLB, these aggregates are located predominantly in neurons and are called Lewy Bodies (LB). These LB are one of the pathological hallmarks of PD and DLB, alongside dopaminergic neuron loss in the substantia nigra. Previous studies have demonstrated the ability of patient-derived LB fractions from PD patients to induce nigral and striatal degeneration of dopaminergic neurons as well as α-synuclein pathology after striatal injections in non-human primates. This specific study aimed to determine the effects of injecting these LB fractions into the cortex of non-human primates. To this end, we inoculated mesencephalic human-derived LB fractions into the prefrontal cortex of baboon monkeys that were terminated one year later. Extensive histochemical and biochemical analyses were performed to evaluate pathological markers known to be affected in LB pathologies. To estimate the effect of cortical injections on dopaminergic production and neuron survival, we characterized the pattern of Tyrosine Hydroxylase (TH), Aromatic L-amino acid decarboxylase (AADC), Dopamine Transporter (DAT), and DARPP-32 staining in the striatum and the substantia nigra of cortical LB-injected monkeys. We also assessed the regional distribution of total and phosphorylated α-synuclein in the prefrontal cortex and the survival of different cell types (i.e., neurons, microglia, astrocytes) after cortical LB injections.Overall, we observed region-specific alterations in several connected brain areas, both in the cortex and striatum. In conclusion, this study provides novel data demonstrating the cortical toxicity of mesencephalic LB extracts in non-human primates.

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Poster

619. Parkinson's Disease: Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 619.03

Topic: C.03. Parkinson's Disease

Title: Silencing ofATP13A2 in monkey substantia nigra induces dopaminergic neurodegeneration and Ser129-phosphorylation of α-synuclein

Authors: J. SIKORA[1,2], S. DOVERO[1], M.-L. AROTCARENA[1], ***E. Y. PIOLI**[3], E. BEZARD[1], B. DEHAY[1], P.-O. FERNAGUT[2];
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Abstract: Lysosomal impairment is strongly implicated in Parkinson's disease (PD). The *ATP13A2* gene encodes a transmembrane lysosomal P5-type ATPase that acts as a lysosomal polyamine exporter. Mutations in the *ATP13A2* gene were identified as the cause of Kufor-Rakeb syndrome (KRS), a juvenile-onset form of PD. In previous studies, we discovered that mutations/deficiencies in ATP13A2 gene lead to systemic lysosomal damage characterized by lysosomal membrane instability, impaired lysosome acidification, decreased processing of lysosomal enzymes, decreased degradation of lysosomal substrates, and clearance of autophagosomes. These defects collectively contribute to α-synuclein accumulation and subsequent cell death.This project aimed to determine the potential to induce PD or KRS pathology in non-human primates through a viral vector-based approach. To this end, we injected bilaterally into the substantia nigra of macaque monkeys a lentiviral vector expressing an ATP13A2 small hairpin RNA. Animals were terminated 5 months later and brains harvested. Extensive histochemical and biochemical analyses were performed to evaluate cerebral pathological markers known to be affected in KRS and PD. We characterized the pattern of dopaminergic loss in the striatum and the substantia nigra, the regional distribution of α-synuclein immunoreactivity in several brain structures, as well as its pathological status (i.e., S129 phosphorylation), the accumulation of heavy metals and occurrence of lysosomal dysfunction. Overall, at 5 months weeks after injection, significant and ongoing degeneration of the nigrostriatal pathway is observed upon lentivirus-mediated ATP13A2 silencing. In conclusion, this study demonstrates that decreased ATP13A2 function leads to dopaminergic neurodegeneration in non-human primates and provides a potential new model for PD research. Additional analyses are ongoing to further understand the role of lysosomal ATP13A2 protein in the pathogenesis of PD and to uncover mechanisms contributing to the occurrence of neurodegeneration observed in the APT13A2 deficient monkeys that might open new therapeutic opportunities for slowing down the degenerative process in patients with KRS or PD.

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Poster

619. Parkinson's Disease: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 619.04

Topic: C.03. Parkinson's Disease

Support: MSA Coalition

Title: Grk2 targeted knock-down as therapy for multiple system atrophy

Authors: *E. BEZARD[1], A. DELAMARRE[2], M.-L. AROTÇARENA[2], M. LOPEZ CUINA[2], W. MEISSNER[3];
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Abstract: Developing neuroprotective and disease-modifying treatments for multiple system atrophy (MSA) is an urgent unmet need. Insulin and related factors act as neuromodulators in the brain. Neurodegenerative diseases are characterized by impaired insulin/insulin-like growth factor-1 (IGF-1) signaling and insulin resistance (i.e., decreased insulin/IGF-1 signaling). We recently demonstrated that such insulin resistance is a crucial feature of (MSA) in the brain of MSA patients and the gold-standard transgenic mice model of MSA, the PLP-synuclein mouse. Counteracting such insulin resistance thus bears the potential of being neuroprotective and improving autonomic failure such as orthostatic hypotension.The insulin signaling is notably transduced by an integrator protein named G protein-coupled receptor kinase 2 (GRK2). Both mouse models of insulin resistance and humans with metabolic syndromes display increased levels of GRK2. Interestingly, a recent attempt to knock down the peripheral expression of GRK2 resulted in a reversal of diet-induced obesity and insulin resistance in a model of diabetes. We first investigated the levels of GRK2 expression in MSA patients' brains. We then tested the hypothesis that knocking down the expression of GRK2 in the whole body (through in utero delivery of AAV- GRK2 miRNA) or specifically in the brain (through intrastriatal delivery of AAV- GRK2 miRNA in adult mice) has positive effects on insulin resistance, dopamine cell survival and α-synuclein pathology in the gold-standard transgenic mice model of MSA, the PLP-synuclein mouse.Down-regulating GRK2 levels in the striatum or brain protect from developing nigrostriatal lesions and synuclein accumulation in the striatum. GRK2 thus appears as a possible target. Beyond gene therapy, small molecules interfering with GRK2 function are worth testing with CNS delivery to avoid unwanted peripheral side effects.

Disclosures: E. Bezard: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac Neuroscience LTD. **A. Delamarre:** None. **M. Arotçarena:** None. **M. Lopez Cuina:** None. **W. Meissner:** None.

Poster

619. Parkinson's Disease: Animal Models

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Program #/Poster #: 619.05

Topic: C.03. Parkinson's Disease

Support: ViCTER

Title: Humanized alpha synuclein overexpression delays neuropathology in a viral model of Parkinsons DIsease

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Abstract: Humanized alpha-synuclein overexpression delays neuropathology in a viral model of Parkinsons DIsease

Casey P. McDermott[1], Collin Bantle[1], Dev Aldaz[2], Savannah Rocha[2], Katriana Popichak[2], Ken Olson[2], Ronald B. Tjalkens[1]

Parkinsons disease (PD) is a movement disorder of unknown etiology. The hallmarks of PD include glial reactivity, neurodegneration in the Substnantia Nigra (SN), and alpha synuclein aggregation. Infection with Western Equine Encephalitic Virus (WEEV) has previously characterized to recapitulate all 3. In this study, we examined how WEEV could propagate neuropathology in transgenic mice overexpressing a human alpha-synuclein variant (A53T). We postulated that infection with WEEV would accelerate alpha-synuclein aggregation as early as 1 month post-infection. Mice were infected with WEEV and neuropathology was examined at 1 and 3 months post. Markers of pathology were assessed in relevant regions by immunofluorescence staining. At 1 month post-infection we see extensive gliosis, but not a loss of dopaminergic neurons. Phosphorylated a-syn was also measured and was only found to be significantly increased in the cortex. By 3 months, gliosis subsided, but significant neurodegeneration was found. This supports the hypothesis that a-synuclein has an antiviral function in the brain.

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Poster

619. Parkinson's Disease: Animal Models

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Program #/Poster #: 619.06

Topic: C.03. Parkinson’s Disease

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Title: Developmental exposure to the Parkinson’s disease-associated organochlorine pesticide dieldrin exacerbates synucleinopathy-induced deficits in dopamine uptake in the striatum in the α-synuclein pre-formed fibril (PFF) mouse model

Authors: S. L. BOYD[1], N. C. KUHN[1], J. R. PATTERSON[1], A. C. STOLL[1], M. R. KOLANOWSKI[2], J. J. NEUBECKER[2], S. A. ZIMMERMAN[2], K. C. LUK[3], E. RAMSSON[2], C. E. SORTWELL[1], *A. I. BERNSTEIN[1];
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Abstract: Parkinson’s disease (PD) is the most common neurodegenerative movement disorder and one of the fastest growing neurological diseases worldwide. This rise in incidence outpaces the rate of aging and is increasing most rapidly in newly industrialized areas, suggesting that

environmental factors, such as decreased smoking and specific environmental toxicants, may contribute to these increases. Epidemiological studies have shown that exposure to the organochlorine pesticide dieldrin is associated with increased risk of PD. In addition, animal studies in our lab and others have identified a link between developmental dieldrin exposure and increased neuronal susceptibility to synucleinopathy in the α-synuclein preformed fibril (PFF) model and MPTP toxicity in adult male C57BL/6 mice. However, the mechanisms mediating this effect remain incompletely defined. Specifically, our previous results show that developmental dieldrin exposure induces a male-specific exacerbation of PFF-induced increases in dopamine (DA) turnover as indicated by an increased ratio of the DA metabolite, homovanillic acid, to DA and motor deficits on the challenging beam at 6 months post-PFF injection. To expand on these results, we hypothesize that the observed changes in DA turnover are due to alterations in DA uptake and packaging at the striatal synapse. To test this hypothesis, female C57BL/6 mice (n=11 per group) were exposed to 0.3 mg/kg dieldrin or vehicle control starting at 8 weeks by feeding (every 3 days) throughout breeding, gestation, and lactation. Developmentally exposed male offspring from independent litters (n=8 per group per endpoint) underwent unilateral, intrastriatal injections of α-syn PFFs via stereotaxic surgery at 12 weeks of age to induce synucleinopathy. Female mice were excluded since no effect of dieldrin on synucleinopathy-induced toxicity was observed in our previous study. At 4 months post-PFF injection, we measured vesicular monoamine transporter 2 (VMAT2) uptake velocity by vesicular ^{3}H-DA uptake assays (n=8 per group). There was no dieldrin-induced change in VMAT2 uptake velocity ipsilateral to the PFF injection site. We also assessed striatal DA release and DA transporter (DAT) uptake by fast-scan cyclic voltammetry (FSCV) in striatal brain slices (n=8 per group). We found no dieldrin-induced change in peak height (DA release), but a dieldrin-induced decrease in tau (DAT uptake) in PFF-injected animals ipsilateral to the injection site. These results support our hypothesis that alterations in DA uptake and packaging contribute to dieldrin-induced exacerbation of synucleinopathy-induced deficits in DA turnover.

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Poster

619. Parkinson's Disease: Animal Models

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Title: Positron emission tomography with [18F]ROStrace reveals increased oxidative stress in mouse models of alpha-synucleinopathy

Authors: *E. GALLAGHER[1], S. RILEY[4], Y. ZHU[4], R. CHRONEOS[4], M. SHELDON[4], H. LEE[2], S. LI[2], K. XU[2], N. KOHLI[4], N. PATEL[4], K. C. LUK[3], R. H. MACH[2], M. J. MCMANUS[4]; [1]Neurosci. Grad. Group, [2]Dept. of Radiology, [3]Dept of Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA; [4]Dept. of Anesthesia and Critical Care Medicine; Ctr. for Mitochondrial and Epigenomic Med., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Parkinson's disease (PD) is a debilitating neurodegenerative disorder characterized in part by the progressive accumulation of insoluble intraneuronal inclusions called Lewy Bodies (LBs). LBs are largely composed of oxidatively modified alpha-synuclein (aSyn), and oxidative stress is thought to be both a mediator and a consequence of aSyn aggregation in PD. Here, we aim to determine whether aSyn-induced oxidative stress can be tracked noninvasively with positron emission tomography (PET) for use as a prognostic biomarker in PD and other synucleinopathies. We have recently validated a novel PET tracer, [^{18}F]ROStrace, that detects increased oxidative stress associated with mitochondrial dysfunction and microglial activation in neurodegenerative disease. In the current study, our hypotheses state 1) that PD-associated mutations in aSyn will induce ROS production by the mitochondria and microglia, which will lead to increased oxidative stress that is detectable by [^{18}F]ROStrace, and 2) that [^{18}F]ROStrace signal in brain will correlate with the progression of aSyn neuropathology. To test these hypotheses, we imaged mice at various stages of aSyn aggregation initiated by either 1) overexpression of the PD-associated, pro-fibrillary A53T mutant form of human aSyn (A53T mice), or 2) intrastriatal injection of pre-formed aSyn fibrils (PFFs). In A53T mice, animals of both sexes were imaged during early (6mo) and middle (12mo) stages of aSyn aggregation, and the PET results were correlated with longitudinal behavioral assessments and aSyn pathology at endpoint. In PFF-injected mice, imaging was conducted at 1-, 2-, and 3- months post-injection (MPI). Current results indicate a positive correlation between aSyn pathology and [^{18}F]ROStrace signal in A53T mice ($n=14$, $p<.0001$), with female A53T animals also showing significantly higher whole-brain PET signal and phosphorylated aSyn expression vs female controls at 12mo ($n=6$-10; $p=.039$). At 2MPI, PFF-injected A53T mice showed a significant increase in whole-brain average [^{18}F]ROStrace signal compared to age- and sex-matched saline-injected controls ($n=8$ animals each, $p=.025$). Furthermore, higher PET signal at 2 MPI was negatively associated with subsequent survival time ($n=4$, $R^2=.97$, $p=.017$), indicating that brain [^{18}F]ROStrace signal may correlate with disease severity. Taken together, the current results demonstrate that noninvasive imaging with [^{18}F]ROStrace can detect increased oxidative stress associated with aSyn neuropathology.

Disclosures: E. Gallagher: None. **S. Riley:** None. **Y. Zhu:** None. **R. Chroneos:** None. **M. Sheldon:** None. **H. Lee:** None. **S. Li:** None. **K. Xu:** None. **N. Kohli:** None. **N. Patel:** None. **K.C. Luk:** None. **R.H. Mach:** None. **M.J. McManus:** None.

Poster

619. Parkinson's Disease: Animal Models

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Program #/Poster #: 619.08

Topic: C.03. Parkinson's Disease

Title: Characterization of a model of Parkinson's Disease using synthetic α-synuclein inoculation to evaluate motor function, synucleinopathy, and dopaminergic neurotransmission.

Authors: C. TORTURO*, K. COX, R. PELTZ, S. MONGIA, J. GRESACK, D. HAVAS, D. BUDAC, *S. RAMBOZ;
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Abstract: Alpha-synuclein is a presynaptic neuronal protein that is linked to Parkinson's Disease (PD) and contributes to disease pathogenesis. We sought to build on previous research on PD models by examining the pathogenic effects of synthetic α-synuclein proteins injected into the striatum in male C57Bl6/J WT mice at 8 weeks of age. Briefly, α-synuclein were inoculated via stereotaxic surgery into unilateral or bilateral mouse striatum. Animals were evaluated using a battery of behavioral tests to assess motor function as well as for markers of α-synuclein and dopaminergic neurotransmission. Striatal inoculation with α-synuclein displayed strong Lewy-body-like pathology with hyperphosphorylated α-synuclein aggregates spreading from the striatum to nearby brain regions. We have also identified histopathological decreases in tyrosine hydroxylase, a key marker of dopaminergic neurons as well as a reduction in striatal DA and metabolite levels. Concluding remarks are pending additional behavioral data analysis. Our goal is to further characterize this model for testing disease modifying therapies for Parkinson's Disease.

Disclosures: C. Torturo*: A. Employment/Salary (full or part-time):; PsychoGenics. **K. Cox:** A. Employment/Salary (full or part-time):; PsychoGenics. **R. Peltz:** A. Employment/Salary (full or part-time):; PsychoGenics. **S. Mongia:** A. Employment/Salary (full or part-time):; PsychoGenics. **J. Gresack:** A. Employment/Salary (full or part-time):; PsychoGenics. **D. Havas:** A. Employment/Salary (full or part-time):; PsychoGenics. **D. Budac:** A. Employment/Salary (full or part-time):; PsychoGenics. **S. Ramboz:** A. Employment/Salary (full or part-time):; PsychoGenics.

Poster

619. Parkinson's Disease: Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 619.09

Topic: C.03. Parkinson's Disease

Support: Huffington Foundation

Title: Knock-in Mice Engineered to Carry Parkinson Disease-causing SNCA Mutation

Authors: *Y. KIM[1,4], J. MCINNES[1,4], Y. LIANG[1,4], S. VEERARAGAVAN[2,4], P. ALBELDA DE LA HAZA[2,4], B. D. W. BELFORT[1,4], A. R. GARZA[1,4], B. R. ARENKIEL[1,4], R. C. SAMACO[2,4], H. Y. ZOGHBI[1,3,4,5];

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Abstract: *SNCA*, the gene that encodes alpha synuclein (α-Syn) is linked to Parkinson's Disease (PD) both based on genome-wide association studies, and because of mutations leading to early onset familial PD. To date, most synucleinopathy mouse models involve overexpression of α-synuclein-encoding cDNA driven by a heterologous promoter. To understand the pathogenesis of α-synuclein driven PD, we hypothesized that a knock-in (KI) of a disease-causing mutation into the *Scna* locus in mice will permit better assessment of regional vulnerability and phenotypic progression. To this end, we generated three different *Scna* point mutation KI mouse models (α-syn^{A30P}, α-syn^{E46K}, α-syn^{G51D}). Among them, α-Syn^{G51D} KI mice show the most difference in motor coordination and gait phenotype starting from 9 months of age. Furthermore, α-Syn^{G51D} mice show detergent insoluble α-Syn protein aggregation at 12-months of age. Interestingly, we noted that both olfactory circuit and cortical neurons were positive for phosphorylated- α-syn^{S129} at 12 months of age. These findings suggest that α-syn KI mutant model mice can replicate some early features of PD pathology and will now permit more in-depth pathogenesis studies.

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Poster

619. Parkinson's Disease: Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 619.10

Topic: C.03. Parkinson's Disease

Title: Characterization of GBA1 E326K mutation in progression of Parkinson's disease and their aspect on α-synucleinopathy

Authors: *S. KWEON[1], S. KIM[2], H. KO[1];
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Abstract: GBA1 mutations are the most common genetic risk factor for development of Parkinson's disease (PD). The loss of catalytic activity of GBA1 as well as reduction of GBA1 protein in certain cellular compartment may increase disease progression. Especially, GBA1 activity in lysosomal fraction may cause severe aspect of developing PD. Several types of GBA1 mutations were already reported that lysosomal dysfunction leads development of neurodegeneration along with more serious phenotypes in PD. In this study, reduction of GBA1 protein levels in E326K mutation led downregulation of GCase activity, thereby inducing accumulation of α-synuclein (α-syn) protein. Importantly, in vitro studies demonstrate that GBA

mutation significantly aggravate the α-synuclein preformed fibrils (PFF)-induce neuronal toxicity and the susceptibility to α-syn PFF in GBA E326K knock-in mutant neurons. To understand the mechanism underlying GBA1 E326K mutation, we collected mouse sample longitudinally, and showed different phenotype based on GBA1 activity, function and increment of gliosis in aged mice, which suggested that disease susceptibility based on their activity and protein expression was highly dependent on single mutation of GBA1 protein. Furthermore, the gut-to-brain α-syn transmission mouse model was applied in GBA E326K mice. The pathological α-syn aggregation and propagation from gut to brain were increased in GBA E326K mutation. Thus, phosphorylation of serine 129 of α-syn highly increased in brain lesion along with loss of dopaminergic neurons, motor, and non-motor behavioral deficits. Taken together, our results suggest that GBA1 deficiency due to E326K mutation has a susceptibility to pathologic α-syn. Mutation in GBA1 protein may have disadvantages in survival of dopaminergic neurons in response to α-syn PFF-induced toxicity both from brain and gut.

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Poster

619. Parkinson's Disease: Animal Models

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Program #/Poster #: 619.11

Topic: C.03. Parkinson’s Disease

Title: Implication of neurovascular alterations in Parkinson’s Disease onset and progression in a human alpha-synuclein overexpression mouse model (line 61)

Authors: L. T. PORSCHEN[1], K. LAU[1,2], F. RICHTER[1,2], ***B. GERICKE**[1,2];
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Abstract: Molecular and cellular changes of the brain microvasculature and associated neurovascular cells are increasingly recognized in neurodegenerative diseases such as Alzheimer’s Disease (AD), Parkinson’s Disease (PD) and Multiple Sclerosis (MS) and may occur at early stages of disease prior to neuronal loss. Key cellular mechanisms associated with PD such as the accumulation of alpha-synuclein (aSyn) accompanied by inflammation and oxidative stress may negatively affect the blood-brain barrier (BBB) function and integrity. In fact, evidence for BBB alterations were found in PD patients. However, the underlying molecular mechanisms and extent of microvascular alterations remain to be resolved in detail. Here we analyzed microvascular alterations on a cellular and molecular level in the progressive human aSyn overexpressing Thy1-aSyn PD mouse model (Masliah line 61) by comparison of two and six months old wildtype (WT) and transgenic (TG) mice at different symptomatic stages. Western blot analysis and immunocytochemistry using isolated brain capillaries from different brain regions (cortex and striatum) revealed endothelial aSyn accumulation in TG mice in both brain regions and differential regulation of transporter/carrier protein expression, that are

described to mediate toxin and aSyn transport across the BBB, respectively. Moreover, endothelial activation including elevated expression of endothelial cell adhesion molecules could be shown. Endothelial activation triggers the release of pro-inflammatory cytokines and metalloproteases that are involved in the degradation of tight junction proteins and the basement membrane in turn influencing BBB integrity in specific brain regions. Finally, BBB permeability in different brain regions was studied by histological staining and dye extrusion assay during aSyn accumulation at different stages of PD. A better understanding of neurovascular alterations in PD expands the knowledge about extraneuronal processes and pathomechanisms of the disease that could lead to the identification of novel pharmacological targets.

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Poster

619. Parkinson's Disease: Animal Models

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Program #/Poster #: 619.12

Topic: C.03. Parkinson’s Disease

Support: NSERC
OGS

Title: Synucleinopathic strains induce distinct pathology in REM sleep behaviour disorder

Authors: *B. J. DUGAN[1], R. LUKE[1], J. J. FRAIGNE[1], J. C. WATTS[2], J. H. PEEVER[1];
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Abstract: *Background:* REM sleep behaviour disorder (RBD) is a parasomnia in which patients exhibit abnormal muscle movement during REM sleep. Following RBD onset, up to 90% of patients develop a synucleinopathic disorder such as Parkinson’s disease (PD) or multiple system atrophy (MSA).Though the relationship between RBD and synucleinopathic disease is strong, RBD is only comorbid with PD in 25-58% of patients, yet presents in almost all patients with MSA. Still, few studies have evaluated RBD in a model of synucleinopathy, and all are in the context of PD. There are currently no studies investigating RBD in a model of MSA or the pathological differences between PD and MSA during prodromal stages. The objective of the current study is thus to explore whether the neural circuitry responsible for REM sleep atonia is differentially affected in animal models of PD and MSA.
Methods: To create murine models of both diseases, TgM83$^{+/-}$ mice (a hemizygous, transgenic model of synucleinopathy) were injected with either PD-like or MSA-like, brain-derived α-synuclein aggregates. These preparations have been previously shown to induce pathologically and behaviourally distinct diseases, resembling injections of MSA patient lysate or lysate from a mouse model of PD. To evaluate disease progression, immunohistochemistry was performed at varying time points to look at pathological α-synuclein accumulation and distribution, particularly in the REM atonia circuit.

Results: After injecting α-synuclein aggregates mimicking either MSA or PD into the REM atonia circuit, both cohorts of mice display distinct disease progression. Over time, MSA-like mice display robust, widespread pathology—inside and outside of the REM atonia circuit—while PD-like mice exhibit slower disease progression and pathology in closer proximity to the initial injection site. Moreover, MSA-like mice begin displaying signs of neurological illness three months after initial injections, while PD-like mice present with minor neurological symptoms after six months. Control injections result in no α-synuclein aggregation or motor symptoms at six months following inoculation.
Conclusion & Significance: Akin to clinical observations, the present data suggests that disease progression and severity in the REM sleep atonia circuit is dependent on disease strain. These results provide mechanistic rationale as to why MSA patients may present with RBD in almost all cases, while only some PD patients are co-diagnosed with RBD.

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Poster

620. ALS Mechanisms and Models II

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Title: Increased S10018, S100A9 and MYL12A and decreased SOD1, C3 and C4BP in peripheral blood mononuclear cells from fibromyalgia patients

Authors: *G. PÉREZ SÁNCHEZ[1], M. T. VEGA-RAMÍREZ[1], J. L. MALDONADO-GARCÍA[1], E. BECERRIL-VILLANUEVA[1], J. M. ELIZALDE-CONTRERAS[2], E. RUIZ-MAY[2], E. ESTUDILLO[3], D. MENDIETA[4], L. PAVÓN[1];
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Abstract: Fibromyalgia (FM) is a disorder with a complex etiology that involves genetic, biological, and environmental factors. It is a chronic disorder characterized by widespread pain, fatigue, and cognitive and sleep disturbances. Inflammation and oxidative stress are two factors closely related to the physiopathology of FM. The aim of this work was to obtain the differential

proteomic profile of patients with FM in order to identify potential biomarkers of this disease. For this first approach, eight healthy volunteers and nine patients with FM who met the inclusion criteria were included. We used tandem mass tag coupled to mass spectrometry (TMT-MS), a semi-quantitative technique, to obtain the differential proteomic profiles of peripheral blood mononuclear cells (PBMCs) from FM patients. TMT-MS allow us to perform a relative quantification between two or more conditions. Among the identified proteins, we found that S10018, S100A9 and MYL12A are upregulated in FM patients, whereas SOD1, C3 and C4BP are down-regulated. FM patients had 2.2-, 2.8-, and 3.6-fold higher levels of S10018, S100A9, and MYL12A, respectively, compared with healthy volunteers. In contrast, SOD1, C3 and C4BP levels were 0.38, 0.68 and 0.58, respectively. S10018 and S100A9 participate in several processes of inflammation. MYL12A phosphorylation along with actin filament formation plays a key role in endothelial barrier disruption, which is a hallmark of acute inflammation. Superoxide dismutase 1 (SOD1) is a homodimeric metalloenzyme that catalyzes the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen. SOD1 plays a key role in oxidative stress and it has been reported altered in several diseases like rheumatoid arthritis and schizophrenia. C3 is the most abundant protein of the complement system and participates in the activation phase; whereas C4BP is a regulatory protein in the early phase of both the lectin and the classical pathway. We consider that these six proteins could be potential biomarkers of FM.

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Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.02

Topic: C.06. Neuromuscular Diseases

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INSERM
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Title: Cross frequency coupling, a new quantitative biomarker in Amyotrophic Lateral Sclerosis

Authors: *C. BENETTON[1], A. LACKMY[2], M. LE VAN QUYEN[3], C. ROUAUX[4], P.-F. PRADAT[5], V. MARCHAND-PAUVERT[6];
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Abstract: An early motor cortex cortical hyperexcitability characterizes both sporadic (sALS) and familial (fALS) forms of Amyotrophic Lateral Sclerosis (ALS). This form of cortical dysfunction precedes lower motor neuron signs, suggesting to be a powerful biomarker promoting ALS early diagnosis. Electroencephalography (EEG) can be an approach to detect and monitor cortical dysfunction through the investigation of the interaction between neural oscillations at different frequencies, known as Cross-Frequency Coupling (CFC) analysis. Precisely, we studied the Phase-Amplitude Coupling (PAC) between slow and fast oscillations, known to be highly dependent on excitation/inhibition (E/I) balance in the brain cortex. Our aim was to investigate whether PAC is altered in ALS patients and if this alteration can represent an ALS cortical dysfunction biomarker. We used a high density EEG recording protocol (74 channels, 4kHz sampling rate, bandpass 0.03-1330Hz), consisting in 5 minutes recording with eyes closed and 5 with eyes open, at rest, on 26 sALS patients (median ALSFRS-r score=40) and a gender- and age-matched group of 27 healthy controls. EEG data were pre-processed to delete artefacts using Independent Component Analysis and filters to select EEG signal below 60Hz. We run a spectra analysis and then we analysed PAC on 5 channels around the cranial vertex (Fz, Cz, Pz, C3, C4 according to the 10-20 system) on 3 frequency pairs: Theta-Gamma (4-8Hz vs. 30-60Hz), Alpha-Gamma (8-15Hz vs. 30-60Hz) and Beta-Gamma (15-30Hz vs. 30-60Hz). To do so, we used the Tort et al. method[2], which measures PAC estimating the mean Modulation Index (MI i.e., mean quantitative estimation of the coupling). Our spectra analysis indicate no dysfunction at the level of single frequency bands. The PAC analysis revealed that MI for Theta-Gamma PAC was significantly decreased in ALS patients compared to controls whether the eyes were closed or opened during recording (ANOVA, $p<0.05$), especially at the level of Fz, Cz, Pz and C3 channels. We also found a link between Theta-Gamma MI and ALS disease progression rate, with MI more depressed in fast than slow progressors, suggesting that the faster the progression rate is, the greater the Theta-Gamma PAC reduction gets (One-way ANOVA, $p<0.001$). No difference between groups was observed in Alpha-Gamma and Beta-Gamma PAC MI. Our results suggest a neural uncoupling likely linked to E/I imbalance at cortical level in ALS, and support the hypothesis that the Theta-Gamma PAC MI extracted from resting-state EEG may serve as new quantitative biomarker of cortical dysfunction during ALS disease progression. [2]Tort ABL, et al. *J Neurophysiol* **104**, 1195-1210 (2010)

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Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.03

Topic: C.06. Neuromuscular Diseases

Support: This work was supported by JSPS KAKENHI Grant Number JP 20K07777.

Title: Saccadic oscillations as a parameter of clinical symptoms in amyotrophic lateral sclerosis

Authors: *K. NAKAMAGOE[1,2], S. MATSUMOTO[1], N. TOUNO[1], I. TATENO[1], N. KAJITA[1], I. MIHASHI[1], H. TAKAHASHI[1], T. SUZUKI[1], S. OOUCHI[1], A. TAMAOKA[1], K. IGARI[1], M. TANAKA[1], K. ISHII[1], T. KOGANEZAWA[2];
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Abstract: [Background] We have focused on both ocular movements and vestibular function with neurological disorders as indicators for estimating the effect of treatment such as vestibular stimulation therapy on balance disorders with dementia patients (Nakamagoe 2021). We reported saccadic oscillations or vestibular impairment in Alzheimer' disease, frontotemporal dementia, and amyotrophic lateral sclerosis (ALS) (Nakamagoe 2015, 2016, 2019, and 2019). Moreover, we found that square-wave jerks (SWJs), classified as saccadic oscillations, were observed in ALS patients with their eyes open in the dark. However, the pathological significance of this phenomenon as a clinical indicator remains unclear.
[Objective] The present study analyzed the characteristics of SWJs in patients with ALS with visual fixation (VF) or their eyes open in the dark without VF. Moreover, we analyzed the correlation between the clinical manifestations and characteristics of SWJs in ALS patients to estimate whether SWJs might be a significant clinical indicator.
[Methods] Fifteen patients with ALS (9 males, 6 females, 65.1±10.4 years) and 18 healthy controls (8 males, 10 females, 70.4±3.8 years) were investigated and compared. Saccadic oscillations without VF were detected as SWJs and measured using an (direct current record). Furthermore, we investigated differences in the frequency of SWJs without VF and characteristics of clinical manifestations, such as Mini-Mental State Examination (MMSE), Frontal Assessment Battery (FAB), grip power, %vital capacity, and Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS).
[Results] In the control group, the frequency of SWJs was significantly higher with VF as compared to without VF ($p<0.05$). Unlike the control group, in the ALS group, the frequency of SWJs was significantly higher without VF than with VF ($p<0.05$). Significantly more SWJs were observed in the ALS group than in the Control group without VF ($p<0.05$). Moreover, the frequency of SWJs without VF was significantly higher with dysarthria than without dysarthria ($p<0.05$). There is a correlation between the frequency of SWJs and %vital capacity ($r>0.05$, $p<0.05$). On the other hand, there were no correlations between SWJs and other evaluation items.
[Conclusions] In healthy individuals, SWJs are generated by VF and suppressed without VF. Conversely, in ALS, SWJs are developed rather than suppressed in the absence of VF. SWJs without VF are a pathological symptom that might derive from brainstem dysfunction from the relationship of SWJs with dysarthria or ventilatory failure in ALS. This work was supported by JSPS KAKENHI Grant Number JP 20K07777.

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Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.04

Topic: C.06. Neuromuscular Diseases

Support: Inserm
Sorbonne Université
CNRS

Title: Brain sensorimotor integration in patients with amyotrophic lateral sclerosis

Authors: A. PREUILH[1], M. PÉLÉGRINI-ISSAC[2], A. LACKMY[1], G. QUERIN[3], P.-F. PRADAT[4], ***V. MARCHAND-PAUVERT**[5];
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Abstract: Muscle spindles are disorganized at early stage of development in SOD1 mouse model of amyotrophic lateral sclerosis (ALS) and both group Ia and II fibers start to degenerate at the same stage than motor axons but with slower progression. In patients, we have evidence for infraclinical sensory defect based on the correlation between the depression of N9 wave induced in peripheral axons by nerve stimulation and altered diffusion metrics (MRI) at the level of the dorsal columns of the cervical spinal cord. On the other hand, cortical components of somatosensory evoked potentials (SEP) can be depressed in about half the patients without any clinical evidence for sensory dysfunctions. Since the clinical tests are not specific to spindle proprioception, we raised the questions that this sensory modality might be specifically altered in ALS patients at early stage of the disease and how these likely altered inputs from muscle spindles are integrated by brain structures. We thus developed a bloc designed functional (f) MRI protocol including periods of rest (about 15 sec.) and of 20-second muscle tendon vibrations that specially activate spindles and we have studied the cortical and subcortical responses in 21 patients with ALS (without any clinical evidence for motor dysfunctions in the hand we stimulated and no clinical sensory defect) and a group of 23 age and gender matched healthy controls. All underwent fMRI during muscle-tendon vibrations applied on the less affected or no affected hand (MRC score ≥ 4) for ALS or on the dominant side for healthy controls. Vibrations (65Hz) were applied over the tendon of first dorsal interosseous (FDI) and extensor digitorum (ED; fingers III-IV) thanks to a MRI compatible pneumatic device. Preliminary analysis of the brain activation maps indicates that the activity on the hemisphere contralateral to vibration was depressed in ALS but enhanced on the ipsilateral hemisphere; activity was particularly increased in cerebellum. These first results suggest that activity in cortical and sub-cortical structures involved in sensorimotor integration are altered in ALS. Hyperactivity might be related to the well-known cortical hyperexcitability and disrupted interhemispheric interaction in ALS which might act as compensatory mechanisms.

Disclosures: **A. Preuilh:** None. **M. Pélégrini-Issac:** None. **A. Lackmy:** None. **G. Querin:** None. **P. Pradat:** None. **V. Marchand-Pauvert:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.05

Topic: C.06. Neuromuscular Diseases

Title: Rapamycin reverts TDP-43 splicing defects and mislocalization in human in vitro models of TDP-43 proteinopathy

Authors: *V. CASIRAGHI[1], C. COLOMBRITA[2], S. SANTANGELO[1], I. MILONE[2], M. N. SORCE[2], V. SILANI[1], A. RATTI[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the presence of aggregates of phosphorylated TDP-43 protein in the cytoplasm of affected neurons. The hypothesized pathomechanism is the loss of TDP-43 nuclear function and the concomitant toxic gain of function of the aggregates. Response to stress and formation of stress granules (SG) have been proposed as initiators of TDP-43 pathological aggregation. We previously showed that mild and chronic oxidative stress by arsenite (ARS) induces formation of both SG and phospho-TDP-43 aggregates in primary fibroblasts and iPSC-motor neurons obtained from ALS patients in association to the accumulation of the autophagy receptor P62. Phospho-TDP-43 aggregates resemble those seen in ALS autoptic brains and are more abundant in C9ORF72 than in TARDBP patients' cells.Aim of our study was to generate a robust and reproducible in vitro model of TDP-43 pathology to be used for drug screening. We induced a chronic oxidative insult in human neuroblastoma SK-N-BE cells by exposure to low doses of ARS for 9-24 hours. Our data showed TDP-43 mislocalization from the nucleus to the cytoplasm in both a dose- and time-dependent manner and increase of the autophagy receptor P62. We also observed a defective splicing activity of TDP-43 towards its target genes UNC13A and POLDIP3, a readout of TDP-43 nuclear loss-of-function, upon chronic ARS treatment. Since autophagy impairment favors TDP-43 pathological aggregation, we first tested the autophagy enhancer rapamycin in our in vitro model of TDP-43 proteinopathy. Rapamycin was capable of rescuing ARS-induced loss of TDP-43 splicing activity on its target genes and of reducing TDP-43 cytoplasmic mislocalization and P62 accumulation. We then tested rapamycin in C9ORF72 patient-derived fibroblasts and iPSC-motor neurons, where its efficacy in rescuing ARS-induced loss of TDP-43 splicing activity was confirmed. Rapamycin also significantly reduced ARS-induced phospho-TDP-43 aggregates and SG formation.In conclusion, we have set up human cell models of TDP-43 pathology in which rapamycin was proved to be beneficial in rescuing chronic oxidative stress-induced alterations in TDP-43 splicing activity and cytoplasmic mislocalization by modulating autophagy. Human SK-N-BE and ALS patient-derived cells chronically treated with ARS can therefore be exploited as valuable in vitro platforms for future drug screening approaches.

Disclosures: V. Casiraghi: None. **C. Colombrita:** None. **S. Santangelo:** None. **I. Milone:** None. **M.N. Sorce:** None. **V. Silani:** None. **A. Ratti:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.06

Topic: C.06. Neuromuscular Diseases

Support: RF-2018-12367768

Title: Modeling a novel N-terminal mutation of KIF5A gene in patient-derived iPSC-motoneurons

Authors: *S. SANTANGELO[1,2], P. BOSSOLASCO[2], C. FALLINI[3], S. MAGRI[4], M. BERTOCCHI[1,2], S. INVERNIZZI[2], D. DI BELLA[4], D. BARDELLI[2], C. COLOMBRITA[2], V. SILANI[5,2], F. TARONI[4], A. RATTI[1,2];
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Abstract: Cytoskeletal and axonal transport deficits are among the primary pathways affected in several neurodegenerative disorders. The Kinesin family member 5A (KIF5A) belongs to a superfamily of microtubule motor proteins involved in the anterograde transport of synaptic vesicles, RNA granules, mitochondria and neurofilaments along dendrites and axons. Interestingly, while loss-of-function *KIF5A* gene mutations in the C-terminal cargo-binding domain are associated with Amyotrophic Lateral Sclerosis (ALS), missense mutations in the N-terminal motor domain are associated with hereditary spastic paraplegia (HSP) and Charcot-Marie-Tooth (CMT2) diseases. We recently identified the novel mutation c.50G>A (p.R17Q) in the ATP-binding motor domain of *KIF5A* gene in a patient diagnosed with HSP.Aim of our work was to model this novel mutation in iPSC-derived motoneurons to unravel the different pathomechanisms associated to *KIF5A* gene mutations in HSP and ALS. We reprogrammed primary fibroblasts from the *KIF5A*-mutated HSP patient into iPSC by Sendai virus kit. The obtained iPSC were fully characterized for the expression of stemness markers and for their pluripotency to spontaneously differentiate into the three germ layers. Karyotype analysis revealed no gross rearrangements during iPSC reprogramming. CRISPR/Cas9 gene editing was used to generate the isogenic *wild-type KIF5A* iPSC line, as well as an iPSC line with a loss-of-function mutation (p.Asn20Lysfs*4) in heterozygous state (*KIF5A*+/-). Whole exome sequencing excluded off-target variants upon gene-editing.The mutant *KIF5A* iPSCs efficiently differentiated into motoneurons, similarly to the isogenic wild type cells, as shown by the expression of typical motoneuronal markers. Western blot analysis revealed a similar amount of KIF5A protein in mutant p.R17Q iPSC-motoneurons compared to the isogenic control, suggesting that the mutation does not cause the protein to aggregate or misfold. As expected, *KIF5A*+/- iPSC-motoneurons showed half amount of KIF5A protein. We observed the appearance of axonal swellings in mutant *KIF5A* iPSC-motoneurons and our current effort is

aimed to assess if the *KIF5A* p.R17Q mutation has any impact on mitochondrial and RNA-binding proteins axonal transport rate by live-cell imaging.In conclusion, we here report of the generation of iPSC-motoneurons from an HSP patient carrying a novel mutation in *KIF5A* N-terminal domain that will be a valuable disease model to further elucidate the different pathomechanisms associated with *KIF5A*-related disorders. (Supported by grant RF-2018-12367768 to FT, VS and AR)

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Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.07

Topic: C.06. Neuromuscular Diseases

Title: Validation of Models for Amyotrophic Lateral Sclerosis with human iPSC Motor derived Neurons

Authors: *L. SCHULTZ, O. H.-U. SCHROEDER, A.-M. KNOSPE, M. WINKLER, K. JÜGELT;
NeuroProof Systems GmbH, Rostock, Germany

Abstract: Amyotrophic lateral sclerosis, ALS, is a fatal disease with not fully understood disease mechanisms. Therefore, phenotypic disease models are needed for the development of new therapies.
The disease occurs in familial and sporadic forms, fALS and sALS. Although only about 10% of cases are familial with a known hereditary origin, they are important for disease modeling. Known fALS forms have mutations in the SOD1, C9orf72, TDP-43, FUS, and other genes. Human-induced pluripotent stem cells with fALS mutations can be differentiated toward spinal motor neurons. They are canonical disease models that reflect phenotypic disease symptoms. Mislocalization of TDP-43 proteins in axons and dendrites is a hallmark of ALS. TDP-43 mislocalizations are present in iPSC-derived spinal motor neurons with C9orf72 mutations but not in wild-type motor neurons.
C9orf72 cultures show increased levels of Poly(GR) dipeptides as a second hallmark.

Disclosures: L. Schultz: A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **O.H. Schroeder:** 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.; NeuroProof Systems GmbH. **A. Knospe:** A. Employment/Salary (full or part-time):; NeuroProof Systems

GmbH. **M. Winkler:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **K. Jügelt:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.08

Topic: C.06. Neuromuscular Diseases

Title: Developing iPSC-derived co- and tri-culture systems to unravel mechanisms that promote neurodegeneration in amyotrophic lateral sclerosis (ALS)

Authors: *D. SOOD[1], J. MCINNIS[1], R. PASSARO[1], B. ZHANG[2], T. HAMMOND[1], S. RODRIGUEZ[1], J. DODGE[1];
[1]Rare and Neurologic Dis., [2]Precision Med. and Computat. Biol., Sanofi, Cambridge, MA

Abstract: ALS is a fatal disease of the neuromuscular system that is due to the degeneration of motor neurons (MNs) in the brain and spinal cord. The onset and progression of ALS involves non-cell autonomous effects between glia and neurons; however, the underlying etiology of glia toxicity remains unknown. Several studies using ALS patient post-mortem spinal cord tissue samples and ALS animal models of disease indicate that lipid dysregulation, increased inflammatory signaling, and aberrant calcium signaling contributes to MN degeneration. Here, we developed co-culture and tri-culture models of disease using SOD1 ALS patient- iPSC derived motor neurons, spinal astrocytes, and microglia to determine whether these systems feature pathogenic phenotypes of ALS. In our co- and tri-culture systems of disease we found that MN neurite outgrowth and area was significantly reduced compared to their isogenic controls, with effects being most prominent when both diseased astrocytes and microglia were present. Preliminary results also show increased lipid droplet accumulation in the diseased SOD1 astrocytes, along with alterations in several enzymes involved in fatty acid metabolism and lipid droplet formation as detected with untargeted proteomics. In addition, in our disease model co-cultures we detected increased release of the proinflammatory cytokines IL-6 and IL-8 in media. Interestingly, in tri-cultures the presence of microglia boosted the release of IL-6 only from SOD1 astrocyte containing cultures. Moreover, single cell sequencing of co-cultures containing SOD1 astrocytes indicate several inflammatory pathway genes as being upregulated. We also measured neuronal activity using genetically encoded calcium sensors to confirm whether SOD1 astrocyte containing cultures exhibit MN hyperactivity as previously reported for MN monocultures. Our overall goal is to develop a human *in vitro* system to model neurodegeneration with robust readouts to aid in the discovery and validation of promising therapeutic targets that alleviate pathogenic features of disease and slow MN degeneration. Towards that goal, we are also working on generating similar *in vitro* models of disease using iPSC cells derived from ALS patients with mutations in TDP-43 and C9orf72.

Disclosures: **D. Sood:** A. Employment/Salary (full or part-time):; Sanofi. **J. Mcinnis:** A. Employment/Salary (full or part-time):; Sanofi. **R. Passaro:** A. Employment/Salary (full or part-time):; Sanofi. **B. Zhang:** A. Employment/Salary (full or part-time):; Sanofi. **T. Hammond:** A. Employment/Salary (full or part-time):; Sanofi. **S. Rodriguez:** A. Employment/Salary (full or part-time):; Sanofi. **J. Dodge:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

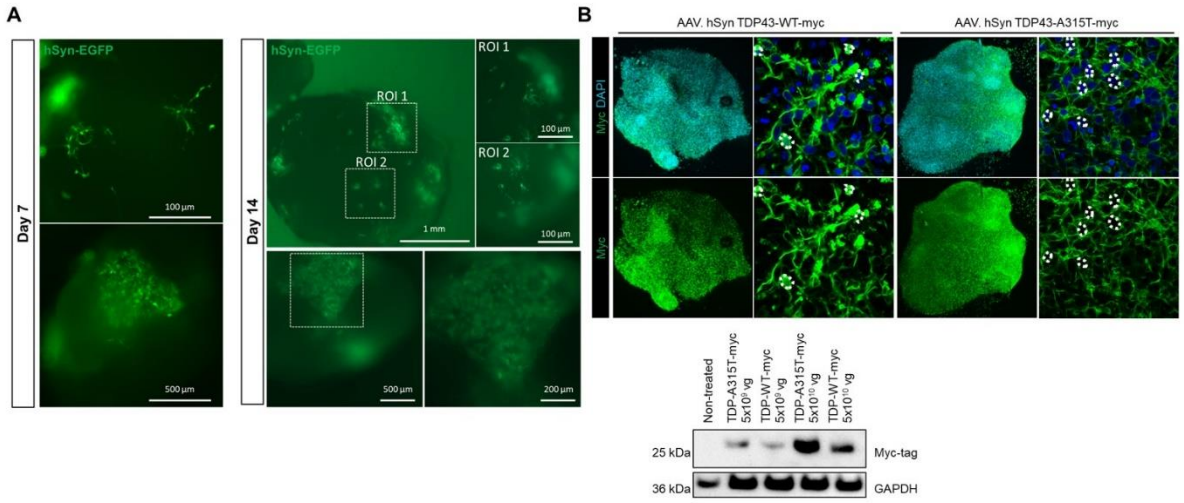
Program #/Poster #: 620.09

Topic: C.06. Neuromuscular Diseases

Title: Neurodegeneration modelling in human stem cell derived-cortical organoids using adeno associated virus (AAV)

Authors: *A.-N. CHO, N. MOREY, F. BRIGHT, Y. KE, L. ITTNER;
Dementia Res. Ctr., Macquarie Univ., Sydney, Australia

Abstract: Frontotemporal dementia (FTD) and Amyotrophic lateral sclerosis (ALS) are rapidly progressing fatal neurodegenerative diseases with similar disease etiology and common neuropathological inclusions containing the nuclear TAR DNA-binding protein 43 (TDP-43). To date, great efforts have been made over several decades, there remains no truly effective treatment available to prevent or cure disease. Major reasons for this high failure are the complex pathophysiology of FTD/ALS, incomplete understanding of underlying disease mechanisms and, foremost, species differences between humans and those animals used for preclinical model. The discovery and development of pluripotent stem cells (PSCs) enabled personalised and patient-specific disease modeling, drug discovery and regenerative therapies. Moreover, recently *brain organoids* that resemble features of human brain are expected to be a major advancement over traditional 2D cultures of neuron, because organoids present cellular organization, specific genetics and molecular pathways reminiscing the human brain. To establish a new model for studying neurodegenerative disease in brain organoid, I expressed TDP-43 on brain organoid via *adeno associated virus (AAV)* delivery with a myc-tag attached to a wild type and mutant TDP-43 under hSynapsin promoter in brain organoid. This successful gene deliveries were confirmed by immunostaining and western blotting. The result showed that mis-localisation of TDP-43 in brain organoids transduced with AAV was triggered in mutant human organoids which infected by AAV hSyn TDP43-A315T-myc, while non-mutant TDP-43 expressed by AAV hSyn TDP43-myc showed normal neurclear distribution. This work suggest the *AAV technique* successfully modeled key features of FTD/ALS by TDP-43 expression model in human organoid which will provide the powerful methodologies opening up novel possibilities to research translation for future development of tailor personalised therapeutics.



Disclosures: **A. Cho:** None. **N. Morey:** None. **F. Bright:** None. **Y. Ke:** None. **L. Ittner:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

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Program #/Poster #: 620.10

Topic: C.06. Neuromuscular Diseases

Support: ICMR grant PGI/IEC/2014/2249

Title: Als patients plasma decreased the viability of nsc34 cells via altering mrna expression of vegf

Authors: *R. KHOSLA[1], A. ANAND[2];
[1]Neurolgy, [2]Neurol., Post Grad. Inst. of Med. Educ. and Res., Chandigarh, India

Abstract: Introduction: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disorder and progressively leads to degeneration of motor neurons at the neuromascular junctions, resulting in paralysis to the patients. The confirm clinical diagnosis of ALS is difficult and takes time that further delays the timely treatment. Changes in plasma composition can be reflected upon CSF composition and hence, can be used to study the diagnosis and prognosis markers for the disease. **Aim:** To develop a simple model system using motor neuron like cell line after induction with plasma. **Method:** Neuroblastoma × Spinal Cord hybridoma cell line (NSC34) was cultured under appropriate conditions. 10% ALS patients' plasma was added to the media and cells were conditioned for 12 hours. Cell survival analysis and differential gene expression of a panel of molecules including VEGF, VEGFR2, ANG, OPTN, TDP43 and MCP-1 was investigated.**Results:** ALS patients' plasma impacted the cell viability and reduced the survival to nearly 50% after induction. VEGF was found to be

significantly down-regulated in the cells which can be explained as a reason for reduced cell survival.**Conclusion:** As CSF induction studies for ALS with NSC 34 cell line are established as model system to study various therapeutic prognosis and diagnosis of ALS. The plasma conditioning can also bring changes in the NSC 34 cells and may be used as model system considering the reflection of changed plasma composition in CSF. However, more studies are warranted in the field. This model system will aid in avoiding the invasive method of collecting CSF from the patients and the controls for the culture studies.

Disclosures: R. Khosla: None. **A. Anand:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.11

Topic: C.06. Neuromuscular Diseases

Support: Maryland Stem Cell Research Fund: 2019-MSCRFD-5093

Title: Enhanced Axonal Regeneration of iPSC-derived Motor Neurons Harboring SOD1 Mutation

Authors: *K. L. MARSHALL, L. RAJBHANDARI, A. VENKATESAN, N. J. MARAGAKIS, M. H. FARAH;
Johns Hopkins Sch. of Med., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease, characterized by degeneration of upper and lower motor neurons that leads to muscle weakness, paralysis, and death, usually 3-5 years following symptom onset. Though dying-back axonopathy is a hallmark of ALS and many other neurologic diseases, the effects of disease-causing mutations on distal axon biology and axonal regeneration are poorly understood. Using human iPSC-derived spinal motor neurons (hiPSC-MN), we investigated the effect of the *SOD1*^{A4V} mutation on axonal regeneration in compartmentalized microfluidic devices, which are powerful tools for studying distal axons and allow us to individually trace axons that are millimeters in length. Mutations in *Superoxide Dismutase 1* (*SOD1*) were the first to be discovered in ALS patients, and *SOD1*^{A4V} mutation stands out for displaying an aggressively progressing disease-course that is lower motor neuron predominant. We therefore chose to compare the axonal regeneration rate of hiPSC-MNs with the *SOD1*^{A4V} mutation to an isogenic corrected control. We performed axotomies, severing axons at the entry point to the axonal compartments, and used time-lapse live imaging and immunofluorescence staining to assess axon length at 24 hours, 48 hours, and 5 days post-axotomy. SOD1$^{+/A4V}$ hiPSC-MNs regenerated axons more quickly than SOD1$^{+/+}$, which was apparent in the mean length of regenerated axons at 48 hours and 5 days post-axotomy. This unexpected result raises the possibility that the *SOD1*^{A4V} mutation might increase expression of regenerated associated genes or produce differences in growth cone

navigation and emphasizes the need to further characterize the effects of distinct disease-causing mutations on hiPSC-MN axon outgrowth and regeneration.

Disclosures: **K.L. Marshall:** None. **L. Rajbhandari:** None. **A. Venkatesan:** None. **N.J. Maragakis:** None. **M.H. Farah:** None.

Poster

620. ALS Mechanisms and Models II

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.12

Topic: C.06. Neuromuscular Diseases

Support: SPRINT-MND/MS

Title: Fast-type motoneurons in SOD1^{G93A} mouse model of Amyotrophic Lateral Sclerosis show progressive increase in frequency of synaptic inputs and hyperpolarisation-activated inward currents

Authors: *A. G. GNANASAMPANTHAN[1], G. B. MILES[2];
[1]Sch. of Psychology and Neurosci., [2]Sch. of Psychology & Neurosci., Univ. St Andrews, St Andrews, United Kingdom

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterised by the deterioration of upper and lower motoneurons (MNs), which leads to paralysis and death. Pathophysiological alterations in MN output have been shown at early postnatal stages in ALS model mice; however, it is unclear whether these changes are due to alterations in synaptic inputs or intrinsic MN properties. Here, we investigated the mechanisms contributing to altered MN output by using whole-cell patch clamp electrophysiology to study MN properties and synaptic inputs to MNs during the first three postnatal weeks in the SOD1^{G93A} mouse model of ALS. Our study targeted delayed firing, fast-type lumbar MNs that are vulnerable and degenerate in ALS. Our results show that the frequency of mixed postsynaptic currents (PSCs) received by MNs is significantly increased in SOD1^{G93A} mice at the second postnatal week. Further analysis using voltage-clamp protocols support that this increase in PSCs is due to greater excitatory input, with no change in inhibitory PSCs. The origin of this increase in PSCs was investigated by measuring miniature PSCs, which were recorded by blocking action potential firing using tetrodotoxin. The frequency of miniature PSCs was increased in fast-type MNs of SOD1^{G93A} mice at week 2, with no changes observed in the amplitude of miniature PSCs. These data indicate pathological changes in presynaptic components of synaptic inputs to MNs. Analysis of MN intrinsic properties, including capacitance, rheobase, persistent inward currents and post-discharge activity, revealed no significant changes in fast-type MNs of SOD1^{G93A} mice. However, a significant increase in the hyperpolarisation-activated inward current (Ih), an important factor driving recruitment, was observed in two-week-old SOD1^{G93A} mice. Overall, early postnatal changes observed in synaptic inputs demonstrate an early role for

synaptic dysfunction in SOD1 ALS. Changes in Ih and synaptic inputs may both contribute to improper MN output, excitotoxicity, and progressive degeneration of spinal MNs in $SOD1^{G93A}$ ALS.

Disclosures: A.G. Gnanasampanthan: None. **G.B. Miles:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.13

Topic: C.06. Neuromuscular Diseases

Support: MND Scotland

Title: Protein methylation as a mechanism for cellular damage in Motor Neuron Disease

Authors: S. BURLEY[1], S. A. SHARPLES[2], C. BONTHRON[2], M. BROADHEAD[2], A. GNANASAMPANTHAN[2], K. MORRISON[1], ***G. MILES**[2], J. SLEEMAN[1];
[1]Sch. of Biol., [2]Sch. of Psychology and Neurosci., Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Amyotrophic Lateral Sclerosis (ALS) is the most common form of adult-onset motor neuron disease and is often characterized by abnormal accumulations of proteins such as FUS and TDP-43. The RNA-binding protein FUS, typically assembles into membrane-less phase separated domains. However, in ALS, FUS is seen to abnormally accumulate in the cytoplasm. One potentially shared disease mechanism across ALS is the dysregulation of post-translational protein methylation. This key, but relatively under-studied, post-translational modification affects the behaviour of proteins including their ability to interact with other proteins and RNAs, as well as their propensity to form phase-separated structures in the nucleus and cytoplasm of cells. The methylation of proteins on arginine residues occurs in three forms: monomethylation (MMA) and symmetrical or asymmetrical dimethylation (sDMA, aDMA). We have observed major defects in the sub-cellular distribution of proteins that usually contain a methylation modification in cells expressing FLAG-tagged FUS-R495X, a causative mutation for fALS. In these cells, methylation-containing proteins co-mislocalise with mutant FUS in cytoplasmic aggregates. The methylated, splicing snRNP protein, SmB also shows altered localisation with a notable loss from splicing speckles, while non-methylated members of the Sm protein family are unaltered. This as well as other evidence suggests alterations to specific forms of post-translational methylation in endogenous proteins and consequent disruption of phase-separated cellular domains. To assess across ALS genetics and ultimately test the importance of the molecular pathways disrupted by altered methylation status we have additionally looked at the ALS mutation *C9ORF72* in iPSC-derived motor neurons incorporating functional studies. We used a combination of pharmacological and viral approaches to manipulate the methylation status of proteins in control and *C9ORF72* mutation harbouring disease lines. Interestingly,

whole cell patch clamp electrophysiology revealed a hypoexcitability-like phenotype characterized by a reduction in the capacity to repetitively fire action potentials that is associated with ion channel dysfunction and also found in *C9ORF72* mutant lines. We additionally observed an unexpected astrocytic disruption when manipulating post-translational methylation. We hope this study will provide a potential unifying molecular defect in cases of ALS of different genetic origins and highlight the importance of pathways affected by alterations in methylation.

Disclosures: **S. Burley:** None. **S.A. Sharples:** None. **C. Bonthron:** None. **M. Broadhead:** None. **A. Gnanasampanthan:** None. **K. Morrison:** None. **G. Miles:** None. **J. Sleeman:** None.

Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.14

Topic: C.06. Neuromuscular Diseases

Support: RCSI STAR PhD Programme (to EJ)
Science Foundation Ireland (17/JPND/3455 and SFI FutureNeuro Research Centre 16/RC/3948 co-funded under the European Regional Development Fund and by FutureNeuro industry partners)

Title: Effects of ALS-associated tRNA-derived stress-induced RNAs on the transcriptomic and proteomic profiles of primary neurons in vitro

Authors: *E. JIRSTRÖM[1,2], A. MATVEEVA[1], P. DONOVAN[1,2], I. ARIJS[3,4], B. BOECKX[3,4], D. LAMBRECHTS[3,4], A. GARCIA-MUNOZ[5], K. WYNNE[5], D. MATALLANAS[5], M. HELM[6], J. H. M. PREHN[1,2];
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Abstract: tRNA-derived stress-induced RNAs (tiRNAs) are a new class of small regulatory non-coding RNAs. tiRNAs are derived from specific tRNA cleavage by the ribonuclease angiogenin as part of an evolutionary conserved stress response. Of note, mutations in the gene encoding angiogenin are associated with several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), indicating that defects in tiRNA production may promote neurodegeneration. Furthermore, recent studies have identified elevated levels of specific tiRNAs, including 5'tiRNA-Gly-GCC, in sporadic ALS patients. However, the biological role of tiRNA production in ALS remains largely unknown. This study aimed to identify the transcriptomic and proteomic

changes induced by 5'tiRNA-Gly-GCC, which is abundantly generated in preclinical ALS models and ALS patients, and to explore biological pathways and functions that may be regulated by this tiRNA in neurons. Cultures of mouse primary cortical neurons were transfected with synthetic mimics of either 5'tiRNA-Gly-GCC or a scrambled tiRNA control. Whole transcriptome RNA sequencing and quantitative label-free LC-MS/MS were performed on the tiRNA-mimic and scrambled-transfected neurons (n=5 per condition) followed by differential expression analysis, functional enrichment analysis and protein-protein interaction (PPI) network studies. Quantitative proteomics analysis identified differentially expressed proteins (DEPs; $p<0.05$, paired moderated t-test) in the 5'tiRNA-Gly-GCC mimic-transfected neurons compared to control, whereas no differentially expressed genes (DEGs; $p<0.05$, DESeq2 Wald test) were identified in response to the tiRNA. Functional enrichment analysis of the proteomic data revealed downregulation of proteins significantly enriched ($p<0.05$, Fisher's exact test) in biological processes and pathways related to translation, gene expression and RNA metabolism. PPI network analysis of the DEPs identified protein clusters associated with proteasomes, ribosomes, spliceosomes and several neurodegenerative diseases, including ALS. Collectively, these findings suggest that 5'tiRNA-Gly-GCC has a substantially stronger impact on the proteome than on the transcriptome in neurons. Thus, we propose that 5'tiRNA-Gly-GCC generated in response to neuronal stress regulates gene expression primarily at the post-transcriptional and translational level. In conclusion, this study provides novel insight into the genome-wide regulation of stress responses by tiRNAs and their potential role in ALS.

Disclosures: E. Jirström: None. **A. Matveeva:** None. **P. Donovan:** None. **I. Arijs:** None. **B. Boeckx:** None. **D. Lambrechts:** None. **A. Garcia-Munoz:** None. **K. Wynne:** None. **D. Matallanas:** None. **M. Helm:** F. Consulting Fees (e.g., advisory boards); Moderna Inc. **J.H.M. Prehn:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.15

Topic: C.06. Neuromuscular Diseases

Support: Department of Defense Grant W81XWH2010161
NIH/NINDS Grant 1R01NS117604-01

Title: P2X7-mediated motor neuron death in an hiPSC model of ALS

Authors: *A. E. JOHNS, A. TAGA, M. O'OBRIEN, K. RUST, C. W. HABELA, N. J. MARAGAKIS;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease whose pathological hallmarks include progressive loss of corticospinal and spinal motor neurons.

Since 90% of ALS cases arise from no known heritable basis, most ALS cases cannot be studied using animal models which has led to significant translational challenges for ALS therapeutics. Motor neurons derived from human induced pluripotent stem cells (hiPSC-MN) provide significant insight into the complex neurobiological determinants of ALS as well as translational benefits in the identification of novel therapeutic targets for a larger proportion of ALS cases. This project uses hiPSC-MN and hiPSC-derived astrocytes (hiPSC-A) from control, sporadic, and familial ALS patients to model a purinergic signaling pathway that we believe contributes to MN death in ALS. Our lab has previously shown that ALS hiPSC-A have significantly increased levels of the gap junction protein connexin 43 (Cx43), and that these levels correspond to significantly increased levels of ATP in the in the supernatants from ALS hiPSC-A compared to controls (Almad et al., 2022). We have shown this to be in part due to increased proportions of Cx43 hemichannels in ALS hiPSC-A compared to controls which when treated with the hemichannel specific blocker Gap19, significantly reduces levels of extracellular ATP. The direct impact of high levels of extracellular ATP on hiPSC-MN survival in the context of ALS has never been investigated so there is potential to identify new substrates implicated in pathogenesis, helping fulfill the critical need for novel pharmacological targets. We demonstrate that treatment of hiPSC-MN with an ATP agonist, BzATP, every third day for two weeks at concentrations of 100 uM and above, leads to significant dose-dependent decreases in MN survival which can be rescued by pharmacologically blocking the ionotropic purinergic receptor, P2X7. We further show that P2X7 activation leads to changes in functional properties of hiPSC-MN including spiking and bursting as measured using multielectrode array electrophysiology, which correspond with changes in intracellular calcium levels measured by live calcium imaging. Additionally, we show that the survival of hiPSC-MN in co-culture over time with ALS hiPSC-A is significantly increased when P2X7 activation is blocked, suggesting that P2X7 antagonism may be neuroprotective during the pathogenesis of ALS. With this project we position P2X7 as a novel druggable target that plays a role in astrocyte-mediated neurotoxicity in both sporadic and familial forms of ALS.

Disclosures: **A.E. Johns:** None. **A. Taga:** None. **M. O'Obrien:** None. **K. Rust:** None. **C.W. Habela:** None. **N.J. Maragakis:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.16

Topic: C.06. Neuromuscular Diseases

Support: RF1NS114128

Title: Investigating the convergence of altered neuronal activity and regulation of the C9orf72 gene locus

Authors: *L. GHAFFARI[1], D. TROTTI[2], A. R. HAEUSLER[1];
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Abstract: The (GGGGCC)n nucleotide repeat expansion (NRE) in the C9orf72 gene (C9) is the most common genetic cause of ALS and FTD. The presence of the NRE is proposed to lead to neurodegeneration in three ways that may not be mutually exclusive: 1) repeat RNA transcribed from can sequester essential RNA-binding proteins, 2) dipeptide repeat proteins (DPRs), which are unconventionally translated through non-AUG repeat-associated (RAN) translation mechanism in both sense and antisense directions, can produce five DPR species; poly(GA), poly(GR), poly(GP), poly(PA), and poly(PR), and 3) loss of C9ORF72 protein from the NRE-affected allele. The precise function of the C9orf72 protein is still a topic of investigation and the genetic regulation of the C9orf72 gene locus in both healthy and disease contexts has not been characterized. Using publicly available ChiP-seq data on the UCSC genome browser, I have identified well-defined activity-dependent transcription factors which bind to the promoter region of C9orf72, such as CREB1 and the AP-1 transcription factor complex. With this in mind, I hypothesize that neuronal activity regulates the C9orf72 gene locus and function of C9orf72. Neuronal activity is a relevant genetic modifier of the C9 gene locus because cortical hyper-excitability is a commonly observed phenotype in ALS patients that typically precedes the onset of lower motor neuron dysfunction. The presence of these activity-regulated transcription factors at the promoter region of C9orf72 indicates the potential for a relationship between neuronal activity and C9 expression products both in healthy and disease states. In order to test the hypothesis that neuronal activity regulates the C9orf72 gene locus, I am using 3 model systems: patient-derived hiPSCs differentiated into glutamatergic cortical neurons, cerebral organoids, and primary rodent cultures. I have used neuronal depolarization and repetitive action potential firing in these model systems and subsequently measured C9 RNA and protein levels. By using pharmacological paradigms to modulate neuronal activity, we have observed variable levels of induction of both C9 RNA and protein in rodent primary cultures and patient-derived hiPSC neurons. Moreover, we are investigating the interaction between C9orf72 protein and lysosomes with and without neuronal activation. The relationship between neuronal activity and C9orf72 gene expression and function has just begun to be elucidated by our laboratory and others. There is a critical need to understand this relationship as it might elucidate novel gene-expression focused therapeutic targets and further reveal the role of C9orf72 protein function.

Disclosures: L. Ghaffari: None. **D. Trotti:** None. **A.R. Haeusler:** None.

Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.17

Topic: C.06. Neuromuscular Diseases

Support: NIH R01NS101986
NIH R37NS057553

Title: A novel function for EXOC2 in regulating GGGGCC repeats toxicity in C9ORF72-ALS/FTD iPSC-derived neurons

Authors: *D. O. HALIM[1,2], G. KRISHNAN[1], S. LEE[1], F.-B. GAO[1,2];
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Abstract: GGGGCC (G_4C_2) repeat expansion in the first intron of *C9ORF72* is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Five different dipeptide repeat (DPR) proteins, poly(GR), poly(GA), poly(PR), poly(GP), and poly(PA), can be synthesized from sense and antisense repeat RNAs. Of these, poly(GR) expression shows a strong correlation with neurodegeneration in *C9ORF72* patient brains. To understand how poly(GR) induces toxicity, we previously performed an unbiased screen in a *Drosophila* model and identified Sec5/EXOC2 as a suppressor of poly(GR) toxicity (*PNAS* 2019). EXOC2 is a subunit of the exocyst complex and involved in protein trafficking and vesicle tethering to the plasma membrane. Here we aim to investigate whether and how EXOC2 modulates neurodegeneration in *C9ORF72* ALS/FTD patient induced pluripotent stem cell-derived motor neurons (iPSC-MNs). Using the CRISPR/Cas9 technology, we generated three *EXOC2* homozygous deletion lines from two *C9ORF72* parental iPSC lines. We showed that *EXOC2* del lines exhibit partial loss of function characterized by reduced neurite numbers and surface NMDAR localization. To determine whether loss of EXOC2 function rescues any ALS/FTD disease phenotypes, we found that *C9ORF72* iPSC-MNs undergo increased axon degeneration compared to isogenic controls upon neurotrophic factor removal and this phenotype was rescued by loss of EXOC2 function. Moreover, three-month-old *C9ORF72* iPSC-MNs have higher apoptotic cell death compared to isogenic controls, which was also rescued by loss of EXOC2 function. To identify the underlying mechanism, we found a decrease in poly(GR) level in *EXOC2* del iPSC-MNs, and surprisingly, the levels of repeat containing *C9ORF72* variants (V1 and V3) were reduced in *EXOC2* del iPSC-MNs whereas the variant without the repeat sequence showed no/modest change. To confirm these observations, we also knocked down *EXOC2* in *C9ORF72* and control iPSC-MNs using antisense oligonucleotides (ASOs). *EXOC2* ASO treatment reduced V1 and V3 mRNA levels in *C9ORF72* iPSC-MNs but not in control iPSC-MNs. *EXOC2* ASO treatment also reduced V1-V3 specific pre-mRNA levels suggesting a transcriptional regulation of expanded G_4C_2 repeats-containing *C9ORF72* allele by EXOC2. Altogether we showed that loss of *EXOC2* function rescues *C9ORF72*-ALS/FTD disease phenotypes by decreasing *C9ORF72* repeat RNA and DPR protein levels. These results identify EXOC2 as a novel regulator of G_4C_2 repeats toxicity in *C9ORF72* iPSC-MNs and reveal a previously unknown novel function of EXOC2 in transcription.

Disclosures: D.O. Halim: None. **G. Krishnan:** None. **S. Lee:** None. **F. Gao:** None.

Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.18

Topic: C.06. Neuromuscular Diseases

Support: E-Rare JTC 2018 project INTEGRALS
Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, via Francesco Sforza 35, 20122, Milan, Italy

Title: Omic characterization of ipsc-derived spinal cord organoids to define new disease-associated genes/pathways in c9orf72 form of amyotrophic lateral sclerosis.

Authors: *N. GALLI[1], M. RIZZUTI[2], L. BRAMBILLA[2], J. ONGARO[2], M. NIZZARDO[2], G. COMI[2], S. CORTI[1];
[1]Univ. of Milan, Milan, Italy; [2]IRCCS Ca' Granda Foundation, Policlinico Hosp. of Milan, Milan, Italy

Abstract: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron (MN) disease with adulthood onset and still represents a prominent health issue, as our knowledge regarding disease pathogenesis is currently lacking. ALS pathophysiology involves progressive MNs degeneration in brain and spinal cord that leads to an irreversible muscle atrophy and death within few years. In the context of multifactorial diseases such as ALS, 3D models are a promising powerful tool that can recapitulate the complex architecture of tissues in a more accurate manner than 2D cultures. The objectives of this work are 1) to characterize induced pluripotent stem cell-derived spinal cord organoids to refine the reliability and reproducibility of the differentiation protocol; 2) to picture the ALS phenotype with an omic approach; 3) to select ALS-related candidate genes/pathways based on the obtained transcriptomic and proteomic profiles. Our spinal cord organoids displayed neural progenitors, post-mitotic neurons, MNs, and glia. In particular, the presence of proliferating neural cells gathered in stemness niches (labeled with SOX2 antibody) decreased in time, indicating organoid maturation. Organoids were collected at 30, 55, and 80 days *in vitro* (DIV) and evaluated for their morphology and neurodevelopmental features by IHC, WB, and qPCR. Specifically, DIV80-organoids expressed SMI32, TUBB3, MAP2, DCX, OLIG2, PAX6, HOXB4, GFAP, and S100β. Besides astrogliosis, the C9 condition interestingly showed PRPH aggregation, as described in literature. We also reported an interesting decrease in Cholinergic Acetyltransferase (ChAT) transcript expression in DIV80-C9 samples. Mass spectrometry and gene ontology depicted an enrichment in pathways related with cytoskeletal coordination, synaptic functionality, astrocyte reactivity, and stress response in C9-ALS condition. Single-cell RNA sequencing and gene annotation disclosed the predominance of neuroectoderm and neural cell populations in the samples, remarking the potential of this disease model. Our omics data are currently under examination, but data obtained will allow the assessment of novel candidate genes and proteins associated with *C9ORF72*-ALS pathogenesis and their potential as therapeutic targets.

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Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.19

Topic: C.06. Neuromuscular Diseases

Title: Defining the functional role of nonsense-mediated decay in healthy and ALS-associated human motor neurons

Authors: *F. ALESSANDRINI[1], M. WRIGHT PALMER[1], T. KUROSAKI[2], L. MAQUAT[2], E. KISKINIS[1];
[1]Northwestern Univ., Chicago, IL; [2]Univ. of Rochester, Rochester, NY

Abstract: Nonsense-mediated decay (NMD) is a highly conserved mRNA surveillance pathway that plays a broad role in cellular homeostasis. It is estimated to target and degrade up to 10% of physiological mRNAs. Typically, NMD engages on transcripts harboring a premature stop codon due to differential splicing, although it can also regulate the expression of normal protein coding genes, the features of which remain poorly defined. The precise role of NMD in neurons remains unresolved although it has been shown to enable compartmentilized gene expression, and to modulate synaptic activity and axon guidance. What also remains unclear is how the pathway is modulated in neurodegenrative diseases with impaired mRNA homeostasis such as Amyotrophic Lateral Sclerosis (ALS). We and others have shown that overexpression of the key NDM factor UPF1 rescues neurotoxicity in several genetic ALS models, including neurons with C9orf72, FUS and TDP-43 mutations, however this mechanism remains controversial. Here, we used spinal motor neurons differentiated from induced pluripotent stem cells (iPSCs) to comprehensively define the role of NMD in this most vulnerable cell type afflicted in ALS patients. We specifically performed RNA-Sequencing in neurons treated with a siRNA for UPF1 and performed gene expression and mRNA stability analysis, in combination with RIP-Seq for the active form of pUFP1. These overlapping datasets highlighted a stringent set of genes that are targeted for degradation by the pUPF1-NMD pathway. To better understand the physiological role of the pathway in post-mitotic neurons we analyzed the features of the target genes not [EK1] harboring PTCs. We find that high GC content within the 3'UTR region is the most prominent feature shared by such mRNAs, while high GC content in the 5'UTR is a characteristic of specific mRNA isoforms. Further, NMD-regulated genes in motor neurons are strongly enriched for function within the ubiquitination-protein degradation pathway, suggesting that NMD plays a regulatory role. Utilizing the stringently defined class of NMD targets we next asked how the pathway is modulated in response to ALS-associated mRNA perturbations. Analysis of RNA-Seq datasets from mutant C9orf72 ALS patient neurons, neurons treated with TDP-43 siRNA and postmortem ALS patient samples suggests that TDP-43 dysfunction subtly impairs the efficiency of NMD-dependent degradation. Our results suggest that TDP-43 dysfunction may burden mRNA surveillance pathways such as NMD. More broadly, our study provides a comprehensive description of NMD activity in post-mitotic cells and offer novel insights into the role of NMD in ALS-associated degeneration.

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Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.20

Topic: C.06. Neuromuscular Diseases

Support: RO1NS094557
RO1AG054025

Title: A11-positive dipeptide repeat oligomers in ALS and FTD

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Abstract: The hexanucleotide repeat expansion in C9orf72 is one of the most common causes of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The hexanucleotide expansion generates toxic dipeptide protein repeats including GP, GA, GR, PA, and PR. Increasing evidence suggests that soluble species, like oligomers, are the main cause of neurotoxicity in neurodegenerative conditions such as Alzheimer's and Parkinson's disease, however, this is unclear in ALS and FTD. Here, we took a reductionist approach and investigated the ability of DPRs to aggregate and form toxic oligomers as well as interact with amyloidogenic proteins like tau. We synthesized short dipeptides repeats (GA, PR, and GR) of varying lengths and used biophysical as well as biochemical assays to characterize the aggregates in vitro and in cellular models. We also used immunoblotting and immunostaining to detect DPR oligomers in C9orf72 associated ALS and FTD patients. Our results suggest the propensity for DPRs, especially GR and PR, to form oligomeric structures that can cause toxicity. We also tested the toxicity of the varying DPR aggregates alone and in the presence of tau protein in cellular models. Moreover, we investigated the presence of DPR oligomers in ALS and FTD human samples and their potential role in disease pathogenesis. Thus, the ability to detect and characterize oligomeric DPRs has great potential to further the understanding of these neurodegenerative diseases and aid in the development of targeted therapeutics.

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Poster

620. ALS Mechanisms and Models II

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Topic: C.06. Neuromuscular Diseases

Support: NRF grant 2022R1C1C1009937

Title: C-abl regulates the pathological deposition of tdp-43 via tyr phosphorylation

Authors: *H. PARK[1], S. LEE[2], H. KO[3], S. KIM[1];
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Abstract: c-Abl regulates the pathological deposition of TDP-43 via Tyr phosphorylation
Hyeonwoo Park[1], Saebom Lee[2,3], Han Seok Ko[3,4,#], and Sangjune Kim[1,#1]Department of biology, Chungbuk National University, Cheongju, Chungbuk 28644, Republic of Korea[2]Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, 34141, Republic of Korea[3]Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, [4]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA[#]Correspondence: hko3@jhmi.edu (H.S.K.), sangjune@chungbuk.ac.kr (S.K.)
Accumulating evidences support that non-receptor tyrosine kinase, c-Abl plays a role in the pathogenesis of several neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Here, we found that TDP-43, which was one of the main proteins comprising pathological deposit in amyotrophic lateral sclerosis (ALS), is a novel substrate for c-Abl. This phosphorylation of tyrosine 43 of TDP-43 led to export of TDP-43 into cytoplasm and increased the formation of G3BP1-positive stress granules. The kinase-dead mutant of c-Abl had no effect on the cytoplasmic localization TDP-43 in SH-SY5Y cells. Moreover, the phosphor-mimetic mutant Y43E of TDP43 expression in primary cortical neurons was also shown to accumulate at the neurite granule. Furthermore, the phosphorylation of TDP-43 at tyrosine 43 promoted the aggregation of TDP-43 and increased the neuronal apoptosis in primary cortical neurons, but not in c-Abl-deficient primary cortical neurons. This study implicates the role of c-Abl in the pathogenesis of ALS.

Disclosures: H. Park: None. **S. Lee:** None. **H. Ko:** None. **S. Kim:** None.

Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.22

Topic: C.06. Neuromuscular Diseases

Support: ALS Canada/Brain Canada Hudson Translational Team Grant
Canada First Research Excellence Fund
CQDM FACs program
CHIR (E-Rare-3)

Title: Modeling ALS using human iPSCs-derived astrocytes

Authors: *V. SOUBANNIER, M. CHAINEAU, L. GURSU, G. HAGHI, A. K. FRANCO FLORES, G. ROULEAU, T. M. DURCAN, S. STIFANI;
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Abstract: Astrocytes are the most abundant glial cell in the central nervous system. Under normal physiological conditions, they function to maintain the appropriate homeostatic environment for neuronal functions. Amyotrophic lateral sclerosis (ALS) is an adult-onset motor neuron disease that can be caused by dominantly inherited mutations, in particular in the gene encoding the enzyme Superoxide Dismutase 1 (SOD1). Initial understanding of ALS suggested that motor neuron degeneration is triggered entirely by cell autonomous mechanisms of neuronal death. A growing body of evidence now suggests an involvement of non-cell autonomous mechanisms of motor neuron toxicity elicited by glial cells. Although animal models have been instrumental in improving our understanding of ALS pathophysiological mechanisms, complementary models based on human cells are needed to further investigate these mechanisms. ALS research can benefit from iPSC technologies, which provide the opportunity to study pathophysiology in cells derived directly from ALS patient. We implemented a robust and reproducible protocol to generate human iPSC-derived spinal cord astrocytes with molecular and functional properties of physiological astrocytes. In ALS, toxicity of astrocytes has been proposed to result from reactive astrogliosis, a process involving morphological and functional changes in astrocytes in response to pathological conditions. Our studies show that astrocytes derived from iPSCs generated from SOD1 ALS-patients cells have several characteristics of reactive astrocytes, from increased proliferative capacity to upregulation of specific neuroinflammation genes. Our results suggest that cellular oxidative stress and in particular nuclear oxidative stress is increased in SOD1 ALS patient-derived astrocytes at an early stage. This nuclear oxidative stress is correlated with activation of DNA damage response at a late stage which may be involved in a concomitant NF-κB activation. These results suggest that human iPSC-derived astrocytes provide a suitable platform for further interrogation of non cell-autonomous neuronal degeneration mechanisms mediated by astrocytes in ALS.

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Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.23

Topic: C.06. Neuromuscular Diseases

Title: Immunocytochemical and electrophysiological characterization under stressors of an induced pluripotent stem cell-derived TARDBP mutated motor neurons with TDP-43 pathophysiology

Authors: *T. SHIRAKAWA[1], S. FLORENCIA[1], A. TOYCHIEV[1], M. OKUYAMA[2], M. TAMURA[1];
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[2]Neurosci. Res. Unit, Mitsubishi Tanabe Pharma Corp., Yokohama, Japan

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive disorder that causes muscle weakness due to motor neuron degeneration. Currently, there is no disease-modifying therapy for this fatal disease. One of the reasons is that ALS is highly heterogeneous, i.e., the age of onset and disease progression are variable among patients attributed to a variety of risk genes. However, despite the high heterogeneity of patients, they share a similar phenotypical feature, which is a deposition of TDP-43 protein in neuronal cell bodies, suggesting that it plays a pivotal role in the pathogenesis of ALS. To examine the mechanisms underlying TDP-43 induced neuronal degeneration, we aimed to identify a phenotypical feature and the vulnerability to physiological stressors of the induced pluripotent stem cell(iPSC) derived motor neurons with a *TARDBP* (TDP-43) gene mutation taken from a patient with ALS. Motor neurons were induced from iPSCs with a *TARDBP* A382T mutation and age-matched healthy control. After culturing for 7-14 days, live-cell imaging analyses showed significant inhibition of neurite elongation and immunocytochemical analyses revealed cytoplasmic mislocalization of TDP-43 in ALS motor neurons. The expression of STMN2, the splicing of which is regulated by TDP-43, was decreased compared to the control neurons. To investigate the impact of stressors on motor neurons with TDP-43 pathophysiology, we applied a few stressors in control or ALS motor neurons including oxidative, endoplasmic reticulum, or osmotic stressors. As a result, we found that ALS motor neurons were more vulnerable to an oxidative stressor, ethacrynic acid. Finally, electrophysiological analyses with a multi-electrode array described that ALS motor neurons showed higher excitability compared to control neurons under a physiological condition as well as an oxidatively-stressed condition, suggesting a link between TDP-43 dysfunction and neuronal hyperexcitability followed by neuronal degeneration. These results indicate that the *TARDBP* A382T mutation recapitulates TDP-43 pathophysiology *in vitro* and oxidative stress is one of the key pathways which accelerates TDP-43-induced cellular damage.

Disclosures: **T. Shirakawa:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Holdings America. **S. Florencia:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Holdings America. **A. Toychiev:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Holdings America. **M. Okuyama:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Corporation. **M. Tamura:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Holdings America.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

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Program #/Poster #: 620.24

Topic: C.06. Neuromuscular Diseases

Title: Tdp-43 dysregulation and stmn-2 mis-splicing upon proteasomal inhibition in potential ipsc-derived neuronal als model

Authors: A. POPALZIJ[1], S. VAN HOPPE[1], S. COMPTE SANCERNI[1], E. DE KRAA[1], D. MAGNANI[1], L. BUTI[1], *F. VERKAAR[1], **M. BSIBSI**[1], A. TURNER[2], T. OOSTERVEEN[2], M. VLAMING[1], M. IOVINO[1], L. RITSMA[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons. Many ALS cases are associated with nuclear-to-cytosolic mislocalization and phosphorylation of the transactivation response DNA binding protein (TDP)-43. This is caused by several mutations such as M337V in the TAR DNA binding protein (TARDBP) gene. In addition, proteasomal degradation is often affected, resulting in aggregation of TDP-43. TDP-43 is involved in many mRNA processes, and its depletion results in mis-splicing of the neuronal growth associated factor, stathmin 2 (STMN-2). This mis-splicing contributes to axonal degeneration. To model the ALS phenotypes in vitro, proteasomal inhibition is often used to trigger TDP-43-associated phenotypes. In this poster we assessed if homozygous or heterozygous CRISPR-edited M337V mutations in induced pluripotent stem cell (iPSC)-derived neurons, alone, or in combination with an MG-132 proteasomal inhibition trigger, would result in TDP-43 phosphorylation and aggregation and/or STMN-2 splicing, and could therefore be used as an ALS-relevant disease model. Immunocytochemistry, high-content imaging, and automated image analysis were used for visualization, localization, and quantification of TDP-43 in individual cells. Here, increase in TDP-43 phosphorylation and aggregation was observed upon acute proteasomal inhibition in both control and M337V mutant cell lines compared to their respective untreated sample. More interestingly, digital droplet PCR showed decrease of full length STMN-2 and the generation of truncated STMN-2 upon acute MG-132 treatment in most cell lines. Additionally, preliminary functional data generated using Multi Electrode Arrays (MEA) showed that all neuronal lines demonstrated formation of synchronous activity at late stage of maturation, suggesting these neurons are electrophysiologically active and amenable to functional studies. These results give more insight in the establishment of iPSC-derived cellular models for ALS using TDP-43 dysregulation and STMN-2 mis-splicing, with its re-expression as a prospective therapeutic approach.

Disclosures: **A. Popalzij:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **S. van Hoppe:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **S. Compte Sancerni:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **E. de Kraa:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **D. Magnani:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **L. Buti:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **F. Verkaar:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **M. Bsibsi:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **A. Turner:** A. Employment/Salary (full or part-time):; Bit.bio. **T. Oosterveen:** A. Employment/Salary (full or part-time):; Bit.bio. **M. Vlaming:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **M. Iovino:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **L. Ritsma:** A. Employment/Salary (full or part-time):; Charles River Laboratories.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.25

Topic: C.06. Neuromuscular Diseases

Support: iMQRES Scholarship 2017534/20191556

Title: Developing a molecular platform for the rapid functional study of novel oligogenic ALS candidate genes in vitro

Authors: *S. WU, J. FIFITA, L. HENDEN, I. BLAIR, S. YANG;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease wherein upper or lower motor neurons degenerate. In 70% of ALS families, the disease is monogenic. Meanwhile, the remaining unsolved 30% display reduced disease penetrance or non-Mendelian inheritance, which are challenging to solve via traditional genetic analyses. Hence, we developed a novel gene discovery strategy, comprising genetic, *in silico* and *in vitro* pipelines, and applied it to MQ1, an Australian ALS family that screened negative for known ALS-causative gene mutations. First, the genetic pipeline filtered 70,300 candidate variants to just 5. Of the 5 candidate variants, 2 candidate variants—A and B—exhibited strong potential for pathogenicity according to both *in silico* and *in vitro* functional pipelines. Furthermore, updating our strategy with new bioinformatics tools allowed us to screen for short tandem repeat (STR) expansions in next-generation sequencing data. This revealed an intermediately expanded *ataxin-2* (*ATXN2*) STR—known to increase ALS risk—in affected MQ1 patients. Interestingly, *ATXN2* and candidate genes *A* and *B* are almost adjacent to each other on the same chromosome, whilst *ATXN2* is located approximately 1 Mb from *A* and *B*. The close genomic proximity suggests co-inheritance of all 3 candidate variants, further supporting oligogenic disease effects in MQ1.To enable the continued use of our rapid *in vitro* functional pipeline, which relies on a transient overexpression paradigm, and overcome low co-expression, we developed a novel molecular multicistronic platform. Co-expression of candidates A and B in HEK293T cells increased 10-fold whilst preserving the phenotypes observed when each candidate variant was studied alone. However, co-expression of both candidate variants A and B was neither toxic nor apoptotic, as assessed via flow cytometry and nuclei fragmentation counts respectively. Using this novel multicistronic platform, we also achieved efficient transient co-expression of MQ1-specific *ATXN2* alleles and candidate variants A and B. When assessed via four color confocal microscopy, there was no difference in cellular morphology between wildtype and MQ1-specific expanded ATXN2. Furthermore, cytoplasmic inclusions were positive for candidate variants A and B, but not ATXN2 regardless of STR size. Taken together, we have developed a promising molecular platform to examine oligogenic effects in ALS and have optimized an *in vitro* pipeline that enables the functional study of multiple genes simultaneously and rapidly.

Disclosures: S. Wu: None. **J. Fifita:** None. **L. Henden:** None. **I. Blair:** None. **S. Yang:** None.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

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Program #/Poster #: 620.26

Topic: C.06. Neuromuscular Diseases

Title: Functional Phenotypic Screening Models for Amyotrophic Lateral Sclerosis with human iPSC-derived spinal motor neurons

Authors: *O. H.-U. SCHROEDER, L. SCHULTZ, A.-M. KNOSPE, M. WINKLER, K. JÜGELT;
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Abstract: Amyotrophic lateral sclerosis, ALS, is a fatal disease with not fully understood disease mechanisms. Therefore, phenotypic disease models are needed for the development of new therapies. The disease occurs in familial and sporadic forms, fALS and sALS. Although only about 10% of cases are familial with a known hereditary origin, they are important for disease modeling. Known fALS forms have mutations in the SOD1, C9orf72, TDP-43, FUS, and other genes. Human-induced pluripotent stem cells with fALS mutations can be differentiated toward spinal motor neurons. They are canonical disease models that reflect phenotypic disease symptoms. In our hands, iPSC-derived spinal motor neurons with a SOD1 D90A mutation and the C9orf72 mutation could be cultivated on microelectrode array plates for more than 30 days. No difference in survivability between diseased and wild-type cells was observed. First electrophysiological activity can be observed after 7 days and lasts for more than 5 weeks. After 14 days in vitro, a reliable hyperexcitation of the disease motor neurons compared to the wild-type neurons can be observed. Hyperexcitation is consistent with the clinical experience of this disease. Riluzole 2 µM applied on days 8 and 11 in vitro with medium change caused a reliable reduction of hyperexcitation after 14 days in vitro in three different models: with C9orf72 and SOD1 D90A pure motor neurons and in a C9orf72 motor neurons co-culture with C9orf72 astrocytes. We demonstrated that these assays are a robust screening method of potential ALS drugs.

Disclosures: O.H. Schroeder: A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof Systems GmbH. **L. Schultz:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **A. Knospe:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **M. Winkler:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **K. Jügelt:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH.

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.27

Topic: C.06. Neuromuscular Diseases

Title: Neuromuscular junction platform by using human induced pluripotent stem cell-derived cells and its application to drug discovery research

Authors: *H. YOSHIDA[1], O. SANO[3], K. ADACHI-TOMINARI[1], T. SUGIMOTO[2], Z. IKEDA[2], H. IWATA[1], K. NAGASAWA[1], Y. TSUJIHATA[1];
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Abstract: Neuromuscular junction (NMJ) is a specialized synapse between motor neuron (MN) and skeletal muscle cells. Uncontrolled muscular activity due to the dysfunction of NMJ was observed in several neuromuscular disease such as amyotrophic lateral sclerosis (ALS), so that the evaluation of drug candidates for neuromuscular diseases in models with pathophysiological NMJ would be valuable. To achieve it, a simple and reproducible in vitro NMJ platform by using human cells would be required. We established a 2D co-culture NMJ model which utilizes MN and skeletal muscle cells derived from human induced pluripotent stem cells. Intracellular Ca^{2+} fluctuations in MN and skeletal muscle cells were successfully monitored by live cell imager, and recorded Ca^{2+} signals through time-lapse imaging were quantified as peak height and AUC by a waveform analysis algorithm. In an ALS-associated NMJ co-culture model, dose-dependent increase of intracellular Ca^{2+} fluctuations were observed by the treatment of a tool compound which is reported to have neuroprotective effect for MN in ALS model. This NMJ model is an effective system for evaluating drug candidates for human neuromuscular diseases.

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Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.28

Title: WITHDRAWN

Poster

620. ALS Mechanisms and Models II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 620.29

Topic: C.06. Neuromuscular Diseases

Support: URI EGRA Grant

Title: Optimizing transfection efficiency in induced pluripotent stem cell derived neurons with amyotrophic lateral sclerosis and frontotemporal dementia

Authors: *A. COLLINS[1], C. FALLINI[2], M. GREGOIRE[2], E. POTTS[1];
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Abstract: Neurons are post-mitotic, presenting a problem for cellular transfection as the lack of cell division keeps the nuclear membrane intact - making it more difficult for neurons to express exogenous DNA. To improve upon this research technique, we used magnetofection to optimize transfection in human induced pluripotent stem cell (iPSC) derived neurons. Magnetofection utilizes magnetic nanoparticles to associate with the DNA, where the nanoparticles get deposited on the neuronal membrane and trigger their endocytosis upon being exposed to a magnetic field. We transfected iPSC derived neurons with a plasmid containing green fluorescent protein (GFP) at a range of concentrations and a fixed ratio between DNA and the Neuromag nanoparticles. Neurons were transfected at different timepoints *in vitro* to determine the optimal age of transfection. The cells were fixed and stained with Neurofilament-H (NF-H) antibody to identify mature neurons, and then were imaged on a widefield fluorescence microscope. The calculated ratio between the cells transfected with GFP and the total number of live cells showed that lower concentrations of transfected DNA produced higher transfection efficiency in iPSC derived neurons than larger concentrations of DNA with optimal efficiency occurring after two weeks *in vitro*. To use this method of transfection in relation to research in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD), we have created two cell lines of iPSCs from patients diagnosed with ALS disease that contain *C9ORF72* repeat expansions - a commonly implicated gene mutation present in ALS disease and FTD. The cell lines were derived with either (1) Neurogenin-2, Islet-1, and LIM Homeobox 3 (NIL) transcription factors to differentiate cells into lower motor neurons, or (2) overexpression of Neurogenin-2 (NGN2) transcription factors to differentiate cells into cortical neurons. Each line and their derivatives also had isogenic cell line controls made that were corrected to contain a normal amount of *C9ORF72* repeats. Cellular transfection can be used as a tool for our disease cell lines to compare cell function and survival in cortical and motor neurons in the presence of ALS and FTD.

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Poster

620. ALS Mechanisms and Models II

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Topic: C.06. Neuromuscular Diseases

Support: DFG Grant 270949263/GRK2162
Förderverein für HSP-Forschung
WI 3567/2-1, 439144457
Tom Wahlig Foundation

Title: Using iPSC-derived neurons to elucidate the role of spastin in store-operated calcium entry

Authors: *T. RIZO[1], L. A. GEBHARDT[2], J. RIEDLBERGER[3], J. WINKLER[4], M. J. M. FISCHER[5], B. A. NIEMEYER[6], B. WINNER[1];
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Abstract: Pathogenic variants in SPAST, the gene coding for spastin, are the single most common cause of hereditary spastic paraplegia (HSP), a progressive motor neuron disorder affecting mainly the axons of corticospinal motor neurons and is characterized by progressive spasticity of the lower limbs. Spastin is a microtubule-severing protein that in addition contributes to the ER-morphogenesis. How spastin dysregulation leads to axonal degeneration is still unclear. Increasing evidence shows that the endoplasmic reticulum (ER) and its interplay with the microtubule network is crucial for effective store-operated calcium entry (SOCE), a cellular process which is triggered by ER-Ca^{2+} store depletion, and requires reshaping of ER and of the ER-resident protein STIM1. Since spastin influences both microtubule network and endoplasmic reticulum structure, we hypothesized that spastin is necessary for the regulation of Ca^{2+} homeostasis via store-operated calcium entry. Here, we show that dysregulation of spastin alters the endoplasmic reticulum structure, alters the transport of STIM1, and has an impact on the Ca^{2+} regulation via SOCE, which is compromised in neurons derived from the induced pluripotent stem cells (iPSCs) of patients with pathogenic mutations in SPAST. Genome editing using CRISPR/Cas9 technology to correct the pathogenic variants in spastin, successfully restored spastin expression levels, Ca2+ regulation, and axonal integrity. Our results show that spastin is a key component in the regulation of Ca^{2+} homeostasis via SOCE and implicates SOCE dysregulation as a pathogenic mechanism in spastin-linked motor neuron disease

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Poster

620. ALS Mechanisms and Models II

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Program #/Poster #: 620.31

Topic: C.06. Neuromuscular Diseases

Support: the Marie Skłodowska-Curie grant agreement No 765704
Fulbright Spain

Title: A fully human induced-pluripotent stem cell derived BBB model: a step closer to personalized medicine

Authors: *A. ARAGON-GONZALEZ[1,2,3], C. D. SOUZA[2], J. R. KOK[2], A. C. SHAW[2], K. C. MEYER[1], P. J. SHAW[2], L. FERRAIUOLO[2];
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Abstract: (1)Background. The blood-brain barrier (BBB) is considered a dynamic and extremely complex vascular interface capable of exerting a vast array of specialized functions. The brain microvascular endothelial cells (BMECs) are the major components of the BBB and the astrocyte end-feet ensheathing the capillary wall are essential components and regulators of this structure [1]. Specifically, as a result of its significant barrier properties, the BMEC interface restricts the uptake of neuro-therapeutic molecules. Furthermore, BBB and astrocyte dysfunction have been reported in MND/ALS [2], but the importance of BBB dysfunction in this disease is still unclear. New tools to generate stem cells derived BMECs, astrocytes and MNs, genetically reprogrammed from human skin fibroblasts have been developed; thus allowing us to generate a patient-specific model of the BBB. **(2)Objectives.** We aim to develop a BBB human ‘in vitro' model to investigate the mechanisms involved in BBB dysregulation in MND and implications for therapeutic approaches where BBB permeability is a key factor. **(3)Methods.** BMECs were co-cultured with i-astrocytes in health and ALS context. To validate the model, BBB markers, permeability and integrity assays were assessed by immunocytochemistry, qRT-PCR and trans-endothelial resistance measurements (TEER). Furthermore, the excitotoxicity of conditioned media from BMECs monoculture and iA co-culture on i-MNs was tested and quantified by measuring the neurite length. **(4)Results.** BMECs were successfully differentiated from ALS and control hi-PSCs cell lines. The obtained BMECs expressed typical BBB markers, including significant dysregulation of transporters (p value <0.05 - 0.0001) when compared with hi-PSCs. Moreover, BMECs monolayer, BMECs/astrocytes co-culture properties were validated in health and disease contexts. **(5)Conclusions.** A completely humanised and patient-specific BBB with BMECs derived from patients has been successfully developed. Additionally, preliminary data are unlikely to prove an intrinsic differentiation impairment in ALS context, however, permeability functional studies are ongoing to verify the presence of BBB dysfunction and associated mechanisms. **(6)References.** 1. Alvarez 2013; 2. Kushner 1991; 3. Lippmann 2012, 2014; 4. Neal 2019**. (7)Acknowledgements.** We would like to thank donors for taking part in this research. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765704 and from Fulbright Spain.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH MH109382

Title: Role of PERK-B associated SNPs in increased risk for HAND pathology

Authors: *S. GHURA[1], S. BOND[2], X. SHI[3], C.-E. AKAY[3], K.-J. SCIUTTO[3];
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Abstract: Antiretrovirals have increased the life expectancy of people with HIV (PWH), however, about 50% present with a spectrum of cognitive, behavioral or motor impairments termed HIV-associated neurocognitive disorder (HAND). At the cellular level, increased ER stress leading to potentially maladaptive unfolded protein response (UPR) has been reported. A downstream event in UPR is the activation of ER stress sensor PERK, which has been implicated in neurodegenerative disorders. Importantly, PERK activation has been shown to induce inflammation, and mild doses of PERK activation have been shown to be protective against a 'second-hit' or stressor, an effect termed hormesis. The gene encoding PERK is known to contain three non-synonymous SNPs in 30% of the general population. These SNPs are genetically linked forming haplotype B (PERK-B) distinct from the more common haplotype (69%), PERK-A. Notably, PERK-B is associated with an increased risk for progressive supranuclear palsy. We have found that PERK-B is associated with worse cognitive global deficit scores in HAND patients. Current literature on the impact of PERK-B SNPs on its activity remains contradictory. We hypothesize that PERK-B has higher kinase activity, potentially predisposing individuals to neuronal dysfunction. We have developed a novel mouse model expressing the PERK-B SNPs. Preliminary data from cell-free PERK kinase assay shows that PERK-B exhibits higher kinase activity. These data are confirmed *in vitro*, but the effect is potentially cell-specific with a pronounced effect observed in glia compared to neurons. Changes in PERK activity are believed to alter the hormetic response to stress. Indeed, PERK-B neuroglia that are pretreated with PERK-activator are more susceptible to death when subsequently treated with NMDA, compared to PERK-A neuroglia using the same paradigm. Basal changes in PERK activity might contribute to changes in PERK-B's hormetic zone. Additionally, PERK-B glia secretes higher levels of IL6 when treated with a PERK-activator but seems to have lower levels of basal IL6. Current studies are focused on understanding the interaction of PERK-mediated hormesis in the context of inflammation in glial cells. Further, we see *in vivo* evidence of increased kinase activity with PERK-B in the cortex, liver, and pancreas. Gross pathology of aged animals reveals subtle changes in inflammatory markers and microbiome, dependent on sex and haplotype. These findings begin to highlight the context and cell type-dependent PERK

regulation of stress responses, specifically in the context of hormesis, and how these processes are modulated by the PERK-B SNPs to augment HAND pathology.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RO1 DA052826

Title: The Impact of Frontline Antiretroviral Therapy in an iPSC-Derived Neuroglial model

Authors: E. NICKOLOFF-BYBEL[1], A. ANGELUCCI[2], K. M. CHRISTIAN[2], C. AKAY-ESPINOZA[3], K. L. JORDAN-SCIUTTO[1];
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Abstract: Despite the use of antiretroviral therapy (ART), people living with HIV (PLWH) still experience neuropathological alterations resulting in neurocognitive decline. While myeloid cell activation leading to persistent neuroinflammation is thought to drive many of these changes, evidence also suggest that antiretrovirals (ARVs) can drive neuronal injury. Studies in rodents suggest ARVs mediate these effects via oxidative stress, glutamatergic dysfunction, and activation of the integrated stress response (ISR); and while these mechanisms are associated with disease states in PWH, mechanistic studies in a human context have lacked models for rigorous testing. Findings in rodent are further limited as they cannot be infected by HIV. Thus, an all-human model in which to assess the impact of ART on CNS health is needed to identify and develop strategies to mitigate these effects. We developed an induced pluripotent stem cell (iPSC) model consisting of glutamatergic neurons (iN) and astrocytes (iAst) to assess the impact of the drugs that constitute Biktarvey® (combination of Bictegravir, Emtricitabine and Tenofovir Alafenamide (cARVs)) on neuronal and glial health and function. Cocultures were treated with 3 concentrations of these cARVs, based on the plasma C_{max} of each drug, for 10 days and assessed for changes in neuronal function via multi-electrode array analysis, inflammatory output via cytokine array, and astrocytic function via glutamate uptake. At the end of the experiment, the cultures were processed for RNA sequencing analysis to assess gene expression changes. Cell death and oxidative stress were also assessed via immunofluorescence microscopy. Our data indicate that at 3X C_{max}, these cARVs induce significant death in iNs but not in iAst. Moreover, cARVs at C_{max} and 3X C_{max} significantly reduce the ability of iAst to uptake glutamate after 10 days of treatment, indicating their ability to alter astrocytic function at clinically relevant concentrations. Preliminary data also indicate cARVs at C_{max} and 3X C_{max} reduce neuronal

firing, indicating neuronal dysfunction in the absence of overt cell death. Current studies building on these findings will address whether oxidative stress or ISR activation may drive these effects. Finally, current experiments are incorporating iPSC-derived microglia into the iN-iAst model to create a triculture system to model productive HIV infection and assess the contribution of ARV-HIV interactions to neuronal dysfunction and inflammation. Together, these models will allow us to better understand how frontline therapeutics may impact CNS health and function and to develop strategies to mitigate these effects in PLWH.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

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Title: WITHDRAWN

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Antiretroviral therapy activates human microglia despite suppression of viral replication

Authors: *C. AKAY-ESPINOZA[1], S. MATT[2], C. MEURICE[3], K. RUNNER[2], H. S. JOHNSON[4], K. L. JORDAN-SCIUTTO[1], P. J. GASKILL[2];
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Abstract: Up to 50% of people with HIV infection continue to experience neurocognitive impairment (NCI) and other neurological disorders despite vastly improved outcomes with antiretroviral therapy (ART). Additionally, ART is increasingly recognized for its inflammatory effects and potential contribution to the persistence of NCI despite effective viral control. An eradicative HIV cure requires the elimination of virus from all tissues, including the CNS, while controlling the adverse effects of ART. Specifically, examining viral persistence and ART in human CNS myeloid cells is challenging, as freshly isolated primary cells, including microglia, have limited availability and rapidly lose key *in vivo* features *in vitro.* Thus, study of HIV infection in CNS myeloid cells, has been mainly limited to laboratory animal and postmortem studies. However, development of effective therapeutic approaches addressing persistent NCI in the context of ART requires models closely recapitulating the key characteristics of specialized cell populations. Therefore, we have developed human induced pluripotent stem cell (iPSC)-derived microglia (iMg) differentiated from adult human fibroblasts as a tractable system for *in vitro* mechanistic studies of HIV infection. Infection of these iMg with two distinct R5-tropic HIV strains, HIV_{ADA} and HIV_{BaL}, induces increasing viral replication over the course of 9-11 days of infection, determined by HIV p24 Alphalisa. This parallels HIV replication dynamics in human monocyte-derived macrophages. Imaging of mock- and HIV_{ADA}-infected iMg shows high levels of cell fusion and giant-cell formation in infected cultures relative to healthy ramified microglia seen in mock-infected cultures. Continual exposure of infected iMg to the plasma C_{max} concentrations of the antiretroviral drugs bictegravir, tenofovir alafenamide, and emtricitabine, which comprise the front-line regimen Biktarvy®, starting three days after infection suppresses viral replication. HIV infection or ART exposure does not alter glutamate levels in iMg culture medium, and infected iMg exhibit an attenuated, albeit distinct, inflammatory signature in the presence of ART compared to the infected iMg not exposed to ART. These results suggest that iMg are a tractable model that more closely recapitulates the key determinants of HIV infection in the CNS, and can be used to dissect the replication dynamics of HIV infection and assess the impact of ART on cellular outcomes such as inflammatory output from microglia.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Cannabinoid receptor 2signaling as a modifier of human immunodeficiency virus-1 infection and inflammation in human iPSC-derived microglia

Authors: *A. STARR[1], K. L. JORDAN-SCIUTTO[2];
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Abstract: Human Immunodeficiency Virus-1 (HIV) infects microglia and brain-resident macrophages, resulting in CNS inflammation and a viral reservoir that persists despite anti-retroviral therapy. Given the evidence that neurons are not directly infected by HIV, it is likely that indirect, myeloid cell-mediated, neuronal damage is a driver of the mood, memory, and cognitive deficits collectively referred to as HIV-associated neurocognitive disorders (HAND). Cannabinoid receptor 2 (CB_2)-specific signaling is known to attenuate pro-inflammatory cytokine secretion from macrophages without exerting the psychoactive effects traditionally associated with cannabinoids, and thus presents an attractive microglial target to intervene in HAND pathogenesis. We infected human, induced pluripotent stem cell (iPSC)-derived microglia and primary human monocyte-derived macrophages with a myeloid-cell tropic HIV molecular clone and observed the effects of CB_2 activation on infection and activation. We found that the CB_2-specific agonist, JWH-133, alters infection kinetics in a dose-dependent manner. Alterations in cell viability, morphology, cytokine secretion, and glutamate metabolism were also measured as representations of myeloid cell activation. Finally, human iPSC-derived glutamatergic cortical neuron cultures were supplemented with supernatants from HIV-exposed and cannabinoid-treated myeloid cells to observe the effects of CB_2 signaling in established models of indirect neurotoxicity. Recently, common microglia-mediated mechanisms have been identified across HAND and other neurodegenerative conditions, suggesting that modifying CB_2 signaling may be applicable in neuroinflammatory states beyond HIV infection.

Disclosures: A. Starr: None. **K.L. Jordan-Sciutto:** None.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH P20 gm103429

Title: Decreasing activation of microglial cells in the presence of pro-inflammatory stimuli: a role for Levetiracetam?

Authors: *S. REYNOLDS, D. DONLEY;
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Abstract: Neuroinflammation occurs in the Central Nervous System (CNS) in response to foreign matter or damage. Neuroimmune mechanisms protect against neurological damage but

can contribute to neurological disease if they become dysregulated. Microglia are CNS-resident immune cells which activate in response to disease-associated stimuli such as amyloid-beta 42 (A**β**;42). The A**β**;;42 peptide is aggregate-prone and associated with onset and progression of Alzheimer's disease (AD). Additionally, the complement proteins, C3 and C1q are dysregulated in chronic neurological diseases such as Alzheimer's disease and acute disorders such as epileptic seizure activity. Epilepsy is often comorbid with AD and A**β**;42 propagates epileptiform discharges. Therefore understanding how anti-seizure treatments impact microglial responses to stimuli such as A**β**;42, C3, and C1q has implications for a variety of neurological diseases. Levetiracetam (LEV) is a common anti-seizure medication that has been shown to decrease markers of neuroinflammation. Further, it has been shown to ameliorate microglial responses to lipopolysaccharide. The goal of this study was to determine the impact of levetiracetam on the inflammatory response to disease-relevant stimuli including A**β**;42, C3, and C1q. We utilized a 2x2 factorial design with the addition of clinically-relevant concentrations of activating stimuli and levetiracetam. Immortalized mouse microglial cells were stimulated with and without levetiracetam then were subjected to functional analysis. We determined that levetiracetam promotes anti-inflammatory mechanisms, including the production of the cytokine interleukin-10. Treatment with levetiracetam also exerts an additive effect with activating stimuli on metabolic activity. Leviteractiam promotes a shift toward mitochondrial ATP production consistent with a decrease in pro-inflammatory activation. These data contribute to our understanding of how levetiracetam affects microglial function in the context of disease where there may be accumulation of A**β**;42 and/or aberrant expression of C3/C1q. These results suggest that decreases in neuroinflammation observed clinically in patients taking levetiracetam may be a combination of both direct and indirect mechanisms. More work is needed to understand the mechanism(s) involved and how levetiracetam may modify the neuroinflammatory environment during disease such as Alzheimer's disease.

Disclosures: S. Reynolds: None. **D. Donley:** None.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Ameliorate neuroinflammation and cognitive impairment in a murine model of cranial radiotherapy using antisense oligonucleotides.

Authors: *E. PREKA[1], S. SANDNER[1], R. ESPOSITO[1], F. KAMME[2], K. BLOMGREN[1], A. FRAGKOPOULOU[1];
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Abstract: Cranial radiotherapy is an important modality in the treatment of high-grade brain tumors, but it is also considered a major risk factor for cognitive impairment in survivors, particularly in children. Previous work of the group uncovered a biphasic inflammatory response after cranial irradiation (IRR) in juvenile mice, the first occurring 6h and the second 2w post-IR. Single-cell RNA seq data analysis of hippocampal samples showed that the latter peak is dominated by upregulation of type I interferon (IFN) pro-inflammatory genes. Hence, we targeted IFN stimulators, like TANK-binding kinase 1 (TBK1), to inhibit their actions and subsequently prevent the downstream inflammatory pathways. Ionis Pharmaceuticals has developed a novel antisense oligonucleotide (ASO) platform, awarded for its application in spinal muscular atrophy in children. To evaluate the effects of Tbk1 knockdown post-IR, a mouse specific ASO was administered icv to P2 pups, which were then cranially irradiated at P21 with a dose of 8 Gy, and brain tissue was collected 2 w later. The TBK1 downregulation was confirmed on gene and protein levels. Our results revealed sex differences post-IR, with females displaying almost 4-fold higher expression of type I IFN related genes compared to males. Some of these genes, like interferon-induced protein with tetratricopeptide repeats 1 (Ifit1), ubiquitin specific peptidase 18 (Usp18) and signal transduced and activator of transcription 1 (Stat1), were close to control levels in the Tbk1-knockdown females. Interestingly, RNA velocity analysis of the single-cell RNA seq data indicated that Stat1 is a putative driver gene. Bulk RNA seq analysis of hippocampal samples from Tbk1 ASO/IRR-treated mice is in progress to characterize the affected pathways. In conclusion, targeting Tbk1 expression, via a specific ASO, can ameliorate the IR-induced inflammation, and thereby reduce neurodegenerative processes and the cognitive decline, particularly in the female population.

Disclosures: E. Preka: None. **S. Sandner:** None. **R. Esposito:** None. **F. Kamme:** None. **K. Blomgren:** None. **A. Fragkopoulou:** None.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIHP20 GM103429

Title: Ferristatin alleviates inflammation-associated oxidative stress in activated cultured mouse microglial cells

Authors: *A. SCOTT, L. GENRY, D. DONLEY;
Harding Univ., Searcy, AR

Abstract: Ferristatin alleviates inflammation-associated oxidative stress in activated cultured mousemicroglial cells. Authors: Alyssa Scott[1,2]; Landon Genry[2]; David W. Donley[2] Affiliation: 1. Department of Chemistry and Biochemistry, 2. Department of Biology; Harding University,

Searcy, AR 72149. Cellular signaling relies on oxidation/reduction (redox) reactions that result in the formation of free radicals. Unregulated buildup of radicals results in oxidative stress, which can damage cells. This phenomenon is particularly pertinent to microglial cells. Microglial cells are phagocytes that reside in the Central Nervous System, and respond to disease-associated stimuli such as amyloid beta (Aβ). Accumulation of such stimuli causes microglial activation that is associated with oxidative stress. Microglial dysfunction, resulting from oxidative stress, has been implicated in the potentiation of neurodegenerative diseases such as Alzheimer's Disease (AD). It is still not completely understood what role free radical signaling plays in microglial-driven inflammation. Therefore, we used immortalized murine microglial cells to study the relationship between free radicals and inflammation. To evaluate this relationship, we treated cells with the spin trap N-tert-butyl-a-phenylnitrone (PBN) in the presence and absence of inflammatory stimuli. Treatment with PBN decreased elevated phagocytosis resulting from Aβ, decreased production of the pro-inflammatory cytokines TNF-α and IL-6, and reduced activity of inducible Nitric Oxide Synthase (iNOS). Together, these results suggest that free radicals may mediate microglial responses to inflammatory stimuli such as Aβ. One possible contributor to these freeradicals is iron, which is elevated in AD patients coincident with Aβ accumulation. However, the relationship between free iron, chronic oxidative stress, and microglial inflammation is still not clear. We found that cells cultured in elevated iron had increased reactive oxygen species (ROS) and modified responses to Aβ. We also determined that ferristatin, a transferrin receptor inhibitor, decreases ROS generation in cultured microglia and decreased iNOS activity, but the complete mechanism of how iron interacts with Aβ is still unknown. More work is needed to elucidate mechanism(s) by which iron contributes to disease-associated inflammatory response in microglia. However, our results indicate that dysregulation of redox-active iron promotes oxidative stress that modulates microglial responses to extrinsic stimuli.

Disclosures: **A. Scott:** None. **L. Genry:** None. **D. Donley:** None.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Redirecting Microglia Phenotype via Inhibition of NFAT1 Ameliorates Deficits in Mouse Models of synucleinopathies

Authors: *C. KIM[1], M. IBA[1], M. SZABO[1], Y. LEE[2], R. A. RISSMAN[3], S.-J. LEE[4], S. YOU[2], M. COOKSON[1], E. MASLIAH[1];
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Abstract: Abnormal deposition of α-synuclein (α-syn) and neuroinflammation are key contributors to the pathogenesis of Parkinson's disease (PD) and other synucleinopathies such as Dementia with Lewy bodies and Multiple system atrophy. We recently demonstrated that Leucine-rich repeat kinase 2 (LRRK2), a PD-associated gene mediated microglial neurotoxic neuroinflammation via modulation of and nuclear factor of activated T-cells 1 (NFAT1) activity in synucleinopathies. Therefore, we evaluated the possibility of NFAT1 inhibition as a new potential therapeutic strategy for synucleinopathies in current study. To investigate the effect of NFAT1 inhibition in the pathogenesis of synucleinopathies, we intraperitoneally delivered cell-permeable NFAT1 inhibitor, 11R-VIVIT into the wildtype and α-syn transgenic mice (α-syn-tg). Administration of 11R-VIVIT decreased the levels of NFAT1 and microglial pro-inflammatory cytokine gene expressions such as Tnfα and Il-6 in the α-syn-tg. However, neurodegeneration of α-syn-tg was inhibited by NFAT1 inhibition in α-syn-tg. In addition, treatment of 11R-VIVIT ameliorated motor learning behavior in α-syn-tg. Furthermore, accumulation of pathogenic forms of α-syn was also significantly reduced by 11R-VIVIT treatment in α-syn-tg. Most interestingly, microglial transcriptome analysis obtained from isolated microglia from wildtype and α-syn-tg treated with either saline or 11R-VIVIT suggested that inhibition of NFAT1 reduced microglial cytokine gene expression, but also ameliorates cell motility and phagocytosis abilities in α-syn-tg. Indeed, inhibition of NFAT1 re-directed pre- and fully- activated microglia to resting microglia, therefore increased microglial phagocytosis and migration, but also decreased microglial pro-inflammatory cytokine gene expressions. Hence, we propose that inhibition of NFAT1 will be a novel therapeutic strategy for Parkinson's disease and related synucleinopathies.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Washington Red Raspberry Commission
USDA Intramural

Title: Raspberry phenolics in serum from supplemented adults reduce inflammatory stress signals in HAPI rat microglial cells, in vitro

Authors: D. R. FISHER[1], F. PICCOLO[1], M. XIAO[2], B. M. BURTON-FREEMAN[2], ***B. SHUKITT-HALE**[1];
[1]USDA-ARS Human Nutr. Res. Ctr. on Aging, Boston, MA; [2]Illinois Inst. of Technol., Chicago, IL

Abstract: Age-related neurodegeneration and behavioral declines have been associated with increases in neuroinflammation and oxidative stress in the brain. Raspberries contain an array of bioactive phenolic compounds that may play a protective role against chronic age-related diseases, as these compounds exhibit potent antioxidant and anti-inflammatory activities. Because the bioactive compounds in foods are different than those found in circulating blood following consumption, we were interested in whether pre-treatment of stressed cells with serum from people fed raspberries might be a valid model system for assessing their anti-inflammatory effects. The effects of acute raspberry supplementation in older (55-70 years old), overweight/obese (BMI 27-35) adults were examined in a double-blind, placebo-controlled, crossover study. Participants consumed a raspberry or placebo drink with a high-fat breakfast challenge. Blood was collected at baseline (0 hour), then again at 2 and 6 hours post-consumption. HAPI rat microglial cells were treated with the serum prior to stressor application with LPS at 200 ng/mL overnight, and expression of nitric oxide, TNFα, COX-2, and iNOS were measured by western blot and ELISA as inflammatory indices. Results showed that microglia treated with serum from participants who consumed raspberry demonstrated reduced LPS-induced neuroinflammation compared to cells treated with placebo in a time-dependent manner ($p<0.05$). For example, with raspberry supplementation, nitrite production was reduced in serum-treated microglia at both 2 and 6 hrs compared to control. Nitrite levels showed the greatest reduction at 2hr post-consumption, but at 6hr started to return to baseline, suggesting that ongoing supplementation may be needed to provide the greatest health benefits. Therefore, berry metabolites, present in the circulating blood, may be mediating the anti-inflammatory effects of dietary berry fruit, and this attenuation of inflammation may be important in age-related health maintenance.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: TCRD109-44

Title: Immunoproteasome inhibition modulates microglia polarization in intracerebral hemorrhage rats

Authors: *W. HU[1], C. LEE[2,3], H. YU[4], H. LIU[4], S. THANGAMEERAN[5], H. HUANG[4], C. LIN[1], C. PANG[2,4,5], H. LIEW[1,2,4];
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Abstract: Objective: Intracerebral hemorrhage induced proteasome over-activation, leading to an exaggeration of the ER stress/proteostasis disturbance and neuroinflammation. In ICH damage tissue, a biphasic role of activated microglia may achieve a spectrum of functional phenotypes, M1 polarization and M2 polarization, to either exacerbate damage or induce repair and regeneration, depending on the different signals received by microglial receptors. We intended to study the influence of immunoproteasome inhibition in ICH-induced microglia activation. **Materials & Methods:** ICH was induced by intra-striatal infusion of collagenase VII-S, and the ONX-0914(LMP-7 specific inhibitor) was microinjected at 1-hours post-ICH. Neuroinflammation and Phagocytosis were examined by RT-qPCR and immunoblotting, respectively. The neurological deficits were evaluated by modified Neurological Severity Scores. **Results:** Immunoproteasomal inhibition significantly increased phagocytosis level (CD68) at day 3 post-ICH accompanied by hematoma volume reduction and neurological improvement. RT-qPCR results showed that M2 polarization markers were increased, while M1 polarization markers were decreased in the immunoproteasome inhibition treatment group. **Conclusion:** Immunoproteasomal inhibition exerts neuroprotective effect via hematoma clearance and anti-inflammation.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Development of an adult rodent microglia cellular model for the characterization of NLRP3 inflammasome pathway small molecule modulators

Authors: *D. TRUDLER, P. ROLZIN, B. BAILEY, J. BLIESATH, D. RAMIREZ, W. NISHIOKA, N. ENGLISH, U. BANERJEE, Y. CHEN, E. CHANG, H. MONENSCHEIN, R. HODGSON, M. PAPAKOSTA;
Takeda Develop. Ctr. Americas, Inc., San Diego, CA

Abstract: Microglia, the resident immune cells of the central nervous system (CNS), contribute to neurogenesis and neuronal survival, as well as neuronal loss. Microglia-related inflammation and microglial dysfunction have been described in various neurological and neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD) (Gordon et al 2018; Hammond et al 2019). The NLRP3 inflammasome is an important component of the CNS innate immune system and mediator of microglia activation in neurodegenerative diseases. Upon activation by pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) such as misfolded proteins, ATP, nigericin, NLRP3 assembles and oligomerizes with adaptor apoptosis-associated speck-like protein (ASC) and pro-caspase-1 to

form multiprotein intracellular complexes, which activate the caspase-1 cascade. Pro-inflammatory cytokines [interleukin (IL)-1β and IL-18] are released, inducing a lytic cell death (pyroptosis), which induces inflammation leading to neuronal death. To interrogate mechanisms of NLRP3 activation, and identify inhibitors of the response, we have developed a cellular platform using rodent cultures from adult mice or rats. We isolated microglia from adult mouse/rat, using CD11b-magnetic beads. We performed extensive analysis of the purity, viability, and quality of the cells after the isolation using flow cytometry, as well as comparison of various culture methods. The cells were then cultured for 5-6 days and were subjected to a variety of functional assays such as cytokine release and phagocytosis to study the response of the NLRP3 inflammasome. We demonstrate that this adult rodent microglia cellular model is an appropriate system to study the NLRP3 inflammasome, as well as other inflammatory pathways. In addition, we used the selective NLRP3 small molecule inhibitor, MCC950 (Perregaux et al., 2001; Collet al., 2015), to inhibit the inflammasome response in these cell cultures. In accordance with previous data, we show that MCC950 is a selective and potent NLRP3 inhibitor, and that our adult rodent microglia cellular model can be used to further characterize NLRP3 inflammasome pathway novel modulators.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cannabinoid receptor 2 enhances respiratory capacity in naive and TLR4-stimulated microglia

Authors: *B. F. OLABIYI[1], E. GEISSMAR[2], A. ZIMMER[1], A. SCHMÖLE[1];
[1]Inst. of Mol. Psychiatry, [2]Inst. of Innate Immunity, Univ. of Bonn, Bonn, Germany

Abstract: Introduction: Over the last decade, studies in the growing field of immunometabolism have sought to characterize metabolic changes that occur in immune cells after activation. Of focus here is the CB2 receptor, because it is expressed in the resident immune cell of the brain, microglia. It plays a crucial role in regulating innate immune responses to inflammatory stimuli. It is known that activated macrophages undergo a glycolytic switch from oxidative phosphorylation which impairs respiratory capacity. However, it remains unknown whether TLR4 stimulation causes microglia to undergo metabolic changes mediated by the CB2 receptor. To investigate this, we characterized the effects of CB2 deletion and pharmacological inhibition on microglia glucose metabolism and inflammatory profile following TLR4 stimulation.**Methods**: Primary microglia cultures obtained from neonatal wildtype (WT) and

constitutive CB2 knockout ($CB2^{-/-}$) mice were stimulated with TLR4 ligand (LPS/IFNγ) at 100ng/ml and 20ng/ml respectively for 16 hours. Bone marrow-derived macrophages (BMDM) generated from 3-4months WT or $CB2^{-/-}$ mice were subjected to the same TLR4 stimulation. The metabolic parameters: oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were analyzed using the Seahorse XF analyzer. Inflammatory cytokines and transcriptional profile were assessed by ELISA and RNA sequencing respectively. Furthermore, WT microglia or BMDM pre-treated with CB2-specific antagonist SR144528 (SR) were stimulated with LPS/IFNγ for 16 hours after which inflammatory and metabolic profiles were assessed.**Results**: Our OCR results showed that CB2 deletion not only improves the maximal respiration in primary microglia subjected to TLR4 stimulation but also in naive microglia after sequential injection of FCCP. Our RNA sequencing data showed downregulated glycolytic genes in TLR4-stimulated $CB2^{-/-}$ microglia compared to the WT. CB2 inhibition with SR144528 decreases cytokine production in TLR4 stimulated microglia but did not produce any significant difference in OCR and ECAR between TLR4-stimulated microglia with or without SR. **Conclusions**: To conclude, our results suggest that CB2 deletion plays a role in improving mitochondrial respiratory capacity in microglia but not in bone marrow-derived macrophages. Even though TLR4 stimulation displayed a glycolytic switch in both WT and CB2 microglia, treatment with SR144528 does not seem to have a significant effect on mitochondrial function in both microglia and BMDM. Future experiments are still needed to fully understand how CB2 interferes with microglia metabolism under inflammatory settings.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

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Program #/Poster #: 621.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NS043277

Title: Microglial reprogramming by Hv1 antagonism protects from inflammatory neurotoxicity

Authors: *D. HERNÁNDEZ ESPINOSA[1], E. AIZENMAN[2];
[1]Neurobio., Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA; [2]Neurobio., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: The precise mechanisms determining the neurodestructive or neuroprotective activation phenotypes in microglia remain poorly characterized. Metabolic changes may be important for microglial phenotype determination. Metabolism, in turn, can be tightly regulated by changes in intracellular pH. We tested whether pharmacological targeting of the microglial voltage-gated proton channel 1 (Hv1), an important regulator of intracellular microglial pH, is critical for reducing inflammatory neurotoxicity while maintaining the neuroprotective

components of activation. Using mouse primary microglia, we established the activation profile in the absence and presence of pharmacological Hv1 inhibition. Lipopolysaccharide/gamma interferon-mediated activation-induced widespread production of proinflammatory mediators, as well as reactive species and phagocytic activity. In co-cultures with rat cortical neurons, the ensuing neurotoxicity was mainly attributable to the release of tumor necrosis factor alpha (TNFα), reactive oxygen species, and zinc. Strikingly, pharmacological inhibition of Hv1 largely abrogated inflammatory neurotoxicity not only by reducing the production of cytotoxic mediators but also by promoting neurotrophic molecule production and restraining phagocytic activity. Importantly, this Hv1-mediated change from a pro-inflammatory to a neuroprotective phenotype was associated with metabolic reprogramming. Finally, Hv1 antagonism not only reduced inflammatory neurotoxicity but also promoted neuroprotection against a separate, excitotoxic injury. Our results strongly suggest that Hv1 blockers will provide an important therapeutic tool against a wide range of inflammatory neurodegenerative disorders.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

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Program #/Poster #: 621.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Funded by Concept Life Sciences Ltd

Title: Direct comparison of multiple sclerosis drugs in the modulation of inflammatory pathways in microglia

Authors: N. MARTIN[1], C. COLVIN[1], B. HALL[1], T. MODEBADZE[1], **C. COOK**[1], *E. MALAVASI[1], M. BINGHAM[2];
[1]Concept Life Sci., Edinburgh, United Kingdom; [2]Concept Life Sci., Chapel-en-le-Frith, United Kingdom

Abstract: Multiple Sclerosis (MS), a progressive demyelinating and neurodegenerative disorder of the Central Nervous System, affects over 2.8 million people world-wide. Neuroinflammation is a key pathological feature of MS, which is characterised by oligodendrocyte pathology, microgliosis, astrogliosis, and infiltration of peripheral immune cells into the brain. While MS is incurable, existing disease-modifying drugs slow disease progression and ameliorate the symptoms. Many of these drugs affect microglia, where they inhibit pro-inflammatory (M1) polarisation and, in some cases, promote anti-inflammatory and neuroprotective (M2) phenotypes. Nuclear Factor kappa B (NFkB), a master signal transducer driving the activation of microglia towards the M1 phenotype, is activated in microglia in MS. Some MS drugs inhibit NFkB signalling in microglia, and many modulate molecular effectors upstream of NFkB, suggesting that NFkB might be a common molecular effector of MS drugs. However, due to the

variety of methods and model systems used to assess the effect of MS drugs on microglial polarisation and NFkB signalling, a direct comparison of their potencies *in vitro* is not possible using existing literature data. Furthermore, to effectively compare MS drugs, it is necessary to consider not only the clinically relevant concentration, but also the proposed active metabolite *in vivo*. In the present study, we directly compare the ability of different classes of MS drugs and their active metabolites, Fingolimod (FTY720), Fingolimod Phosphate (pFTY720), Dimethyl Fumarate (DMF), Monomethyl Fumarate (MMF), Teriflunomide (TFD), Evobrutinib (EVO) and Tolebrutinib (TOL) to modulate inflammatory phenotypes in microglia. Primary microglia are isolated from Sprague Dawley rat neonate brains, and to induce pro-inflammatory polarisation, cells are treated with lipopolysaccharide (LPS) with or without Interferon-γ (IFN-γ) in the presence or absence of clinically relevant concentrations of FTY720 or pFTY720 (0.1, 1 and 10 µM), DMF (1, 10, 30 µM), MMF (1, 10, 30 µM), TFD (1, 10, 50 µM), EVO (0.01, 0.1, 1, 10 µM) and TOL (0.01, 0.1, 1, 10 µM). After 24 hours of treatment, the supernatants are collected and levels of pro- and anti-inflammatory cytokines (TNFα, IL1β, IL6, IL10, IL12p70, IL4, TGFβ, IL13) analysed by multiplex ELISA. Concomitantly, cells are fixed and stained for microglial marker Iba1 and pro-inflammatory polarisation marker iNOS. Additionally, levels of total and phosphorylated NFkB are measured in treated microglia by Homogeneous Time Resolved Fluorescence. Comparative data for the different drug classes are presented and the impact on NFkB signalling is discussed.

Disclosures: **N. Martin:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **C. Colvin:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **B. Hall:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **T. Modebadze:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **C. Cook:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **E. Malavasi:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **M. Bingham:** A. Employment/Salary (full or part-time):; Concept Life Sciences.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.16

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Tri Beta Award

Title: Sex differences in peripheral neuroinflammation; relief using a novel NSAID nanotherapeutic

Authors: *B. S. DEAL[1], R. VICHARE[2], C. CRELLI[2], L. LUI[2], L. REYNOLDS[3], K. PHILLIPS[1], C. PATTERSON[1], J. JANJIC[2], J. A. POLLOCK[1];
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Abstract: Given the current opioid epidemic that the United States faces today, the call to create a novel, non-opioid analgesic is of great importance. This fact, along with the fact that females have been underrepresented in neuropathic pain studies, propelled this research to evaluate the efficacy of a novel cyclooxygenase 2 (COX-2) inhibiting, nonsteroidal anti-inflammatory nanotherapeutic and its ability to relieve both male and female neuroinflammation and hypersensitivity. The COX-2 inhibitor, Celecoxib, was formulated with the near-infrared (NIR) fluorescent dye for detection in live animals and tissues. *In vitro*, nanotherapeutic pharmacological assays were conducted to evaluate sex differences. Phagocytosis of the nanotherapeutic and its anti-inflammatory effects were assessed using ELISA in both male and female macrophages. *In vivo* assessment explored the effectiveness of the nanotherapeutic in rats experiencing chronic constriction injury (CCI) of the sciatic nerve. The CCI surgery causes both males and female rats to develop quantitatively identical hypersensitivity. This hypersensitivity correlated with the influx of CD68+ macrophages in the fasciculated nerve. Once treated with a single microdose of the COX-2 inhibiting nanotherapeutic, both males and females experienced significant relief from hypersensitivity. However, females' relief was attenuated to only half that as males and lasted only one day while males were relieved multiple days. Even though both male and female neuroinflammation of the sciatic nerve experienced significant decreases in the number of infiltrating macrophages, females were seen to have significantly more than males. The presence of other inflammatory cells was evaluated during neuroinflammation and following treatment. Interestingly, some immune cells, such as T cells, may be influencing the neuroinflammatory response from adjacent tissue rather than from within the fasciculated nerve. These results show that both sexes respond to injury and perceive pain equivalently. Despite this, identical treatment of COX-2 inhibition provides relief from hypersensitivity that was distinct between males and females. Our findings emphasize the need to clarify the mechanistic sex differences in inflammation and in relief from hypersensitivity and pain-like behavior.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Bay-117082-driven nlrp3 inflammasome inhibition resolves nitro-glycerine neuronal damage in in vivo model of migraine

Authors: *M. LANZA[1], A. FILIPPONE[2], G. CASILI[3], I. PATERNITI[3], R. BASILOTTA[3], E. ESPOSITO[4];
[1]CHIBIOFARAM, [2]ChiBioFaram, [4]Dept Chem. Biol. Pharmaceut. and Envrn. Scences, [3]Univ. of Messina, Messina, Italy

Abstract: BAY-117082-driven NLRP3 inflammasome inhibition resolves nitro-glycerine (NTG) neuronal damage in *in vivo* model of migraineAlessia Filippone[1], Sarah Adriana Scuderi[1], Rossella Basilotta[1], Marika Lanza[1], Giovanna Casili[1], Valentina Bova[1], Irene Paterniti [1*] and Emanuela Esposito[1].Migraine is a common neuronal disorder characterized by recurrent episodes of headache associated with a higher prevalence in women than men. Several risk factors have been associated with migraine disease as genetic factors, gender and age. Despite the poor understanding of migraine pathophysiology, it has been reported that NOD-like receptor protein 3 (NLRP3) inflammasome pathway overactivation can contribute to migraine progression. Therefore, the aim of this study was to investigate the effect of BAY-117082, an NLRP3 inflammasome inhibitor, in a mouse model of nitroglycerin (NTG)-induced migraine. The in vivo model of migraine was induced by intraperitoneal (i.p) injection of NTG (dose of 10 mg/kg). Mice were treated intraperitoneally with BAY-117082 at doses of 1 mg/kg, 5 mg/kg, and 10 mg/kg, 5 minutes following NTG injection. After 4 h of NTG injection, the whole brain tissue with the rostral spinal cord were collected and used to perform further analysis. Our results demonstrated that BAY-117082 treatment at higher doses of 5 mg/kg and 10 mg/kg reduced pain attacks, hyperalgesia and photophobia more in female mice NTG-induced. Moreover, the treatment with BAY-117082 significantly reduced histological damage in the trigeminal nerve nucleus in female mice accordingly to significantly decreased in NLRP3 complex components expression levels such as ASC, IL-1β, IL-18, caspase-1 and TNF-α levels. Additionally, the treatment with BAY-117082 at both higher doses significantly modulated CREB/Erk/Akt pathways strictly correlated to the modulation of neurotrophic factors. Taken together, obtained results confer new insight into the role of the NLRP3 inflammasome pathway in migraine pathogenesis, suggesting that BAY-117082 could be considered a novel strategy therapeutics for migraine treatment despite unconventional drug use.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Oklahoma Health Research Program (HR 18-033)
Oklahoma State University Center for Health Sciences, Intramural funds (RLD)
GPSGA grants (SM)

Title: B-funaltrexamine protects against neuroinflammation and behavioral impairment induced by lipopolysaccharide

Authors: *S. MYERS, D. BUCK, K. MCCRACKEN, J. CURTIS, R. L. DAVIS;
OSU-CHS, Tulsa, OK

Abstract: Inflammation is present in a multitude of neurological disorders. For this reason, targeting inflammation has emerged as a viable option for the potential treatment of neurological disorders. Previous work indicated that beta-funaltrexamine (β-FNA), a selective mu-opioid receptor (MOR) antagonist, not only inhibited inflammatory signaling *in vitro* in human astroglial cells but also inhibited lipopolysaccharide (LPS)-induced neuroinflammation and sickness-like behavior in mice when administered immediately post-LPS. The present study explores the extent to which β-FNA is protective when treatment occurs 10 h after LPS administration. Male and female C57BL/6J mice were administered LPS (0.83 mg/kg, i.p.) followed by treatment with β-FNA (50 mg/kg, i.p.) immediately or 10h post-LPS. Sickness- and anxiety-like behavior were assessed using open-field and elevated plus-maze tests, followed by the collection of the whole brain, hippocampus, frontal cortex, cerebellum/brain stem, and plasma. Levels of inflammatory chemokines/cytokines (interferon γ-induced protein, CXCL10; monocyte chemotactic protein 1, CCL2; interleukin-6, IL-6; interleukin-1β, IL-1β, and Tumor Necrosis Factor Alpha, TNF-α) in tissues were measured using enzyme-linked immunosorbent assays. Two-way analysis of variance revealed that at 24 h, LPS increased chemokine and cytokine levels, and β-FNA treatment was protective depending on the dosing schedule and brain region/tissue. β-FNA inhibited levels of CXCL10 in the hippocampus, frontal cortex, cerebellum/brain stem, and plasma, and more so in males. CCL2 levels in the frontal cortex, cerebellum/brain stem, and plasma were differentially affected between males and females. β-FNA also differentially affected IL-6, IL-1β, and TNF-α levels in a region-specific and sex-specific manner. β-FNA effects on sickness- and anxiety-like behavior also differed between males and females. This study suggests that β-FNA treatment may provide neuroprotection in a region-specific manner and the impact is largely sex-dependent. Further examination of β-FNA's anti-inflammatory and neuroprotective actions is still necessary.

Disclosures: S. Myers: None. **D. Buck:** None. **K. McCracken:** None. **J. Curtis:** None. **R.L. Davis:** None.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.19

Title: WITHDRAWN

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation A2018169S
NIH BP-ENDURE R25

Title: Antisense Oligonucleotide-mediated TREM2 Reduction in Tauopathy Mice Regionally Alters Phosphorylated Tau in the Absence of Microglia Activation

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Abstract: Microglia-driven neuroinflammation, along with amyloid-beta plaques and hyperphosphorylated neurofibrillary tangles, contributes to Alzheimer's disease (AD) pathology and facilitates neuronal damage. Genetic variants of microglial genes, including TREM2, have been shown to increase AD risk. TREM2 encodes a microglial receptor, which mediates microglial transition from a homeostatic state to an activated, neurodegeneration-associated state. Though TREM2-deficient tauopathy mouse models suggest that TREM2 deletion reduces neuroinflammation, TREM2 haploinsufficiency exacerbates taupathology and reduces microglial response to injury. However, it is unclear what short-term reductions in TREM2 reveal about its role in tau pathology. We used a mouse-specific TREM2 antisense oligonucleotide (ASO) and acutely reduced TREM2 gene expression in mutant tauopathy mice after the onset of pathology. One month later, brain tissues were probed for phosphorylated tau (p-tau), microglial activation markers, and proinflammatory cytokines. Although there were no statistically significant differences in p-tau in the total hippocampus or its subregions, ASO mediated TREM2-lowering appearedto reduce p-tau within the dentate gyrus and CA3 hippocampal subregions but promote p-tau in the CA1. Surprisingly, microglial activation (evidenced by CD68, Spp1, and other genes) and microgliosis (Iba1 reactivity) were unaltered by TREM2 reduction, suggesting microglial activation is independent of region-specific tau pathology. Our results reveal the complexity of TREM2 as a neuroinflammatorygatekeeper and could inform a region-based therapeutic approach to reducing tau pathology.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: IBS-R001-D2
NRF Grant 2014R1A1A1004857

Title: Inhibiting peripheral and central MAO-B ameliorates joint inflammation and cognitive impairment in rheumatoid arthritis

Authors: *W. WON[1], H.-J. CHOI[2], J.-Y. YOO[2], D. KIM[1], T. KIM[1], Y. JU[1], K. PARK[3], H. LEE[3], S. JUNG[4], C. J. LEE[1];
[1]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Daejeon, Korea, Republic of; [2]CHA Univ., Seongnam, Korea, Republic of; [3]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [4]CHA Bundang Med. Center, CHA Univ., Seongnam, Korea, Republic of

Abstract: Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronic inflammation and the destruction of joints and systemic organs. RA is commonly accompanied by neuropsychiatric complications, such as cognitive impairment and depression. However, the role of monoamine oxidase (MAO) and its inhibitors in controlling neurotransmitters associated with these complications in RA have not been clearly identified. Here, we report that peripheral and central MAO-B are highly associated with joint inflammation and cognitive impairment in RA, respectively. Ribonucleic acid (RNA) sequencing and protein expression quantification were used to show that MAO-B and related molecules, such as gamma-aminobutyric acid (GABA), were elevated in the inflamed synovium of RA patients. In primary cultured fibroblast-like synoviocytes in the RA synovium, MAO-B expression was significantly increased by tumor necrosis factor (TNF)-α-induced autophagy, which produces putrescine, the polyamine substrate for GABA synthesis. We also observed that MAO-B-mediated aberrant astrocytic production of GABA was augmented by interleukin (IL)-1β and inhibited CA1-hippocampal pyramidal neurons, which are responsible for memory storage, in an animal model of RA. Moreover, a newly developed reversible inhibitor of MAO-B ameliorated joint inflammation by inhibiting cyclooxygenase (Cox)-2. Therefore, MAO-B can be an effective therapeutic target for joint inflammation and cognitive impairment in patients with RA.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Faculty of Medicine Ramathibodi Hospital, Mahidol University (NS)
National Research Council of Thailand (PV)

Title: Oxyresveratrol reduces manganese-induced interleukin-8 secretion from macrophages and astrocytes

Authors: *N. SIRIJARASWAN[1], R. SETTACOMKUL[2], P. WOONFAK[2], P. JUTABHA[2], P. VIVITHANAPORN[2];
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Abstract: Manganese passes the blood-brain barrier and accumulates in the brain, especially in the basal ganglia. Excessive exposure to manganese leads to neurological dysfunction referred to as manganism, a Parkinsonian-like syndrome. Manganese causes neuroinflammation by inducing cytokine release from microglia. The prolong inflammatory activation results in neuronal death and astrocyte activation. The present study hypothesized that oxyresveratrol, an anti-inflammatory reagent derived from *Morus alba L*. (mulberry) or *Artocarpus lacucha*, could reduce manganese-induced IL-8 expression from human monocyte-derived macrophages, U-937, and human astrocytoma cells, U-87 MG. Cell viability and levels of IL-8 produced were measured at 24 hours by MTT and ELISA, respectively. Manganese up to 500 µM, oxyresveratrol at 50 µM, and co-treatment of manganese and oxyresveratrol were not cytotoxic to U-937 and U-87 MG cells. Manganese at 50 and 100 µM increased IL-8 secretion of U-937 cells by 1.4 and 2.0 folds, respectively. Oxyresveratrol at 50 µM reduces manganese-induced IL-8 secretion by 37.8 and 46.1% compared with U-937 cells exposed to manganese at 50 and 100 µM, respectively. On the other hand, manganese at 250 and 500 µM increased IL-8 secretion of U-87 MG cells by 7.4 and 15.4 folds, respectively. Oxyresveratrol at 50 µM reduces manganese-induced IL-8 secretion by 42.5 and 64.9% compared with U-87 MG cells exposed to manganese at 250 and 500 µM, respectively. Our results show that oxyresveratrol could reduce inflammation from macrophages and astrocytes induced by manganese. Thus, oxyresveratrol could be developed as an alternative medicine to treat manganese-related health problems.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.23

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Inhibition of type 1 Interferon signaling is beneficial in neuroinflammatory disease

Authors: *C. C. HONG[1], B. VIENGKHOU[2], K. HENRY[3], T. FANG[3], M. J. HOFER[4], F. KAMME[5];
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Abstract: Type I Interferon (IFN I) signaling is a known neuroinflammation pathway that is upregulated in numerous CNS diseases including stroke, traumatic brain injury, and Alzheimer's disease. Genetic knockouts that abrogate interferon signaling have been shown to be beneficial in mouse models of disease, but there are currently no approved therapies that specifically target IFN I signaling in the brain. Centrally-administered antisense oligonucleotides (ASOs) are a clinically translatable modality that has been shown to suppress target RNA in a safe and efficacious manner. Here we use ASOs against Ifnar1, a critical subunit of the sole receptor for type I interferons, to ask whether we can dampen IFN I signaling and improve pathology in a mouse model of cerebral type I interferonopathy (GIFN mice), as well as in normal aged mice. Mice received a single intracerebroventricular (ICV) bolus injection of Ifnar1 mouse-specific ASO. Target knockdown was assessed by qPCR on cortex tissue, and sustained knockdown was observed up to 7 weeks post-ICV. In both GIFN mice and normal aged mice, transcriptome profiling showed that genes in the IFN I pathway were downregulated in Ifnar1 ASO treated groups relative to those treated with control ASO. GIFN mice over-express IFNa, which leads to pathological calcifications and T-cell influx in the brain that are reflective of the human disease. ICV administration of IFNAR1 ASO reduced brain calcifications and T-cell influx in GIFN mice. Our findings demonstrate that Ifnar1 ASO inhibits IFN I signaling in the brain, and ameliorates pathology in the GIFN mouse model of cerebral type I interferonopathy. Type I IFN signaling is prevalent in a wide array of neurological diseases. Ifnar1 ASO is an important tool to evaluate the functional role of this signaling pathway in disease.

Disclosures: **C.C. Hong:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **B. Viengkhou:** None. **K. Henry:** 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. **T. Fang:** 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. **M.J. Hofer:** None. **F. Kamme:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Royal Society Newton Advanced Fellowship NA140170
Flair Fellowship Programme PLR/R1/190829
The University of Cape Town
The South African National Research Foundation
The Blue Brain Project
The Wellcome Trust
The Oppenheimer Memorial Trust

Title: Investigating the anti-inflammatory properties of cestode products in an ex vivo mouse model of neurocysticercosis.

Authors: *A. DE LANGE, A. N. AWALA, T. J. STEYN, R. DANGAREMBIZI, J. V. RAIMONDO;
Human Biol., Univ. of Cape Town, Cape Town, South Africa

Abstract: Neurocysticercosis is a disease in which larvae of the tapeworm *Taenia solium* infect the central nervous system of humans. The disease can be asymptomatic for several years. The onset of symptoms, most commonly seizures, is associated with an intense inflammatory host response surrounding the larvae. Contrastingly, during the asymptomatic phase inflammation is largely absent, presumably due to immunomodulation of the host response by live larvae. To explore the immunomodulatory ability of viable Taenia larvae, we exposed mouse hippocampal organotypic brain slice cultures, to the whole cyst homogenate of a model parasite, *Taenia crassiceps* (*T. crassiceps*). The whole cyst homogenate was applied either on its own or together with an established immunogenic agent, lipopolysaccharide (LPS). The effect of the larval products on innate host immunity was assessed by the measurement of inflammatory cytokines in culture medium using ELISAs, as well as by immunofluorescent co-staining of microglia and astrocytes with the inflammatory transcription factor, NF-IL6. For all experiments a sample size of $N \geq 6$ brain slices was utilized. Based on distribution of data, appropriate parametric or non-parametric statistical tests were utilized. Our results illustrate that whole cyst homogenate did not elicit a significant increase in the release of select proinflammatory cytokines (IL-6 and TNF-α) when compared to untreated control brain slices, nor did it significantly increase NF-IL6 transcription in microglia or astrocytes. Contrastingly, when *T. crassiceps* whole cyst homogenate was applied to brain slices in combination with LPS, the extract was able to strongly and significantly suppress LPS-induced production of the pro-inflammatory cytokines, as well as to significantly reduce the number of NF-IL6-positive microglia and astrocytes. Exploratory snRNAseq data reveals transcriptomic changes that may shed some light on the molecular mechanism by which the larval whole cyst homogenate suppresses innate inflammatory host immune responses. This study makes valuable contributions towards understanding immune modulation by cestode larvae and could further potentially inform the development of a novel, central-nervous-system-specific anti-inflammatory agent.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.25

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH

Title: Alternaria Alternata Inhalation changes brainstem glutamate metabolism and decreases excitation in the nucleus tract solitary in a sex-specific manner

Authors: *P. DA SILVA FROST[1], J. M. VALDEZ[1], K. YISRAEL[1], S. KAMARA[1], K. MKRTCHYAN[1], T. BHAIJEE[1], T. A. BIDDLE[1], D. C. ESTRADA[1], R. DROVER[1], D. D. LO[1], M. J. CARSON[2];
[1]Univ. of California, Riverside, Riverside, CA; [2]Div. of Biomed. Sci., Univ. of California Riverside, Riverside, CA

Abstract: The lungs are directly connected to the brain via vagal nerve afferents, that send chemosensory information (changes in inflammation, immune cell infiltration) to the Nucleus Tract Solitary (NTS), which relay to other brain regions within the brainstem that further control breathing (Botzinger and pre-Botzinger) and the inflammatory response of peripheral organs. The mechanisms in which the central nervous system plays a role in the development of asthma and other respiratory issues remains to be investigated. Here, we examine whether particulate inhalation of Alternaria Alternata one of the most common allergic respiratory triggers, cause changes to the brainstem. Nanostring highthroughput gene expression technology was used for assessing the metabolic and inflammatory profile of the region. Principal component analysis shows males and females have a different metabolic profile at baseline and alternaria inhalation shifted male brainstem gene expression closer to the profile of female control. Genes associated with Glutamate metabolism show an overall downregulation in male and upregulation in female after alternaria exposure, a similar regulation in reactive oxygen response pathway is also observed. Analysis of synaptic targets in the brainstem shows a decrease in excitatory marker glutamate transporter 2 (vglut2) in males and females, without altering synaptic numbers in the region. Analysis of the region which vglut2 is decreased shows NTS decreases vglut2 and synaptophysin co-localized puncta in females only, without changes to breathing regions Botzinger and pre-Botzinger. Collectively, the data suggests lung alternaria inhalation causes glutamate alterations in the brainstem in a sex-specific manner.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.26

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: GZYYGJ2021047
DL2021110001L
2021YFE0201000

Title: Ginsenoside Rb1 attenuates lipopolysaccharide-induced chronic neuroinflammation in mice by tuning the glial cells polarization

Authors: *M. TANG[1], Y. LIU[2], Y. LIU[2], X. WANG[2];
[1]Beijing Univ. of Chinese Med., [2]Beijing Univ. of Chinese Med., Beijing, China

Abstract: Objective: To evaluate whether Ginsenoside Rb1 (Rb1) can attenuate lipopolysaccharide (LPS)-induced chronic neuroinflammation in mice and to explore its relations to glial cells polarization. Methods: In this study we developed a chronic neuroinflammation model in mice by intraperitoneally injection of LPS with an escalating dose. The starting dose was 1 mg/kg, and the injection interval was 48-hour with a 2 mg/kg dose increment each injection with 7 times in total. Once LPS being started, 10 or 20 mg/kg Rb1, or vehicle, was administrated for 14 consecutive days. At the end of treatment, open field test (OFT) and beam walking test (BWT) were used to monitor the behavior changes. Serum cytokines by ELISA were used to monitor the systemic inflammation. The glia cells polarization was investigated through analyzing molecules specific to each glial cell phenotypes. Results: Mice in LPS group had reduced spontaneous activities and impaired beam walking performance. Rb1 obviously ameliorated LPS-induced behaviors disturbances. The serum cytokines show that both tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) significantly increased, while interleukin-10 (IL-10) and transforming growth factor β (TGF-β) remarkably decreased after LPS. Rb1 treatment significantly attenuated LPS-induced serum cytokines changes. RT-PCR and Western Blot showed that TNF-α, iNOS, arginase-1 (Arg-1), and complement component 3 (C3) in the brain significantly increased after LPS, suggesting an extensive neuroinflammation. Rb1 significantly inhibited LPS-induced inflammatory molecules increase. Glial cells polarization analysis showed that both M1 and M2 microglia increased after LPS. The A1 astrocytes was also increased, while A2 astrocytes decreased. Rb1 reduced M1 and M2 microglia, and A1 astrocytes, while significantly increased A2 astrocytes. Conclusion: Rb1 can attenuate chronic neuroinflammation induced by LPS in mice, which may partially attribute to its fine tuning the microglia and astrocytes polarization. The study suggests that Rb1 has potential application value in the treatment of neurodegenerative diseases.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.27

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Imaging Supported Evaluation of Sustained Analgesic and Anti-inflammatory Effects of Theranostic Nanoemulsions in Inflammatory and Nerve Injury Pain Models in Rodents

Authors: ***L. LIU**[1,2], R. VICHARE[1,2], C. CRELLI[1,2], R. MCCALLIN[1,2], M. L. HERNEISEY[1,2], B. DEAL[3,2], K. PHILLIPS[3,2], M. SALEEM[3,2], J. NICHOLS[4], A. J. SHEPHERD[4], J. A. POLLOCK[3,2], J. M. JANJIC[1,2];
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Abstract: Acute neuropathic pain (ANP) poses a significant personal and economic burden. With the present opioid abuse epidemic encompassing all sectors of society, it is pertinent that new therapeutic modalities are developed to effectively treat and resolve ANP. Neuroinflammation is recognized as one of the primary drivers of acute neuropathic to chronic pain transition, leading to prolonged disability and loss of productivity. A major limitation of current non-opioid and local analgesics is their lack of molecular and cellular targeting. Immunomodulating theranostic pain nanomedicines offer unique advantages over standard non-opioid treatments by directly engaging these targets and resulting multifold increase in efficacy. Previously, in rodent models of inflammation and injury, we demonstrated the broad applicability of multimodal (NIRF/^{19}FMRI) nanoimaging agents for visualizing inflammation and establishing a direct correlation between inflammation severity and subsequent pain. The presented pain nanomedicines are theranostic: including both a therapeutic (natural products and/or NSAIDs) and diagnostic (imaging) components. The optimized size and surface properties enable rapid recognition and phagocytosis by circulating macrophages and helps to monitor macrophage infiltration in rodent pain models, both neuropathic and inflammatory. Specifically, we show the effects of novel theranostic nanoemulsions, as imaging supported pain nanomedicines, on the immune pathology in the chronic constriction injury and inflammatory rodent models. The anti-inflammatory efficacy is demonstrated by assessing the expression of macrophage inflammation and pain specific targets (e.g. IL-6, COX-2, TNFα). We also investigated the proportion of M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages in response to treatment in both models. Near-infrared fluorescent imaging is used to monitor macrophage infiltration changes in response to the delivered therapeutic in real time in living animals. Live imaging findings correspond to changes in inflammatory signature in targeted tissues, both in inflammatory pain model and nerve injury rat model. Based on presented findings we propose that theranostic pain nanomedicine platform has the potential to serve as an effective theranostic tool for the treatment and study of acute inflammatory and nerve injury associated pain. The presented pain nanomedicines provide a single, long-lasting, low-dose, non-opioid means of pain relief at the point of injury and/or disease a through targeted immunomodulation, supporting neuromuscular recovery and rehabilitation in multiple pain states.

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Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.28

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Modulatory roles of Trans-cinnamaldehyde on corticohippocampal axis in Wister rat models of long-term sleep deprivation and formalin exposure: a potential therapeutic intervention in dementia

Authors: *J. I. ENYA[1], M. A. AMADI[1], D. P. FINEBONE[1], S. C. SECUNDUS[1], H. B. AKPAN[1], S. O. ELIJAH[1], I. ONYELEONU[1], E. A. ESOM[2,1];
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Abstract: Background: Dementia is a progressive cognitive diminution impeding with normal daily activities that is constantly on the increase. The neurotoxic role of sleep deprivation (SD) and formalin exposure (FEx) in industries and the medical profession has become a thing of concern. **Aim:** This study characterized the corticohippocampal brain axis and examine the neuroprotective effects of trans-cinnamaldehyde (TCA) in Wistar rat models of long-term SD and FEx exposure. **Methods:** Twenty-one healthy male Wistar rats (weighing=140g-150g) were randomly assigned into four groups (n=7). **A-** CON (0.5 ml of sesame oil), **B-** SD+FEx (sleep deprived and exposed to 20% formalin for 8hr, 5 days/week, from day 1 to 60) and **C-** SD+FEx and then TCA (50 mg/kg, from day 30 to 60). 20% formalin exposure was done through whole-body inhalation while TCA was given intraperitoneal. **Results:** SD+FEx exposure significantly led to neurobehavioral impairments, significantly increased MDA, TNFα and IL6, and also significantly reduced SOD, GSH-Px, dopamine, glutamate and acetylcholinesterase levels in brain homogenate. Histomorphometrically, ERK1 gene activation led to oxidative stress, inflammation, and apoptosis (increased IBA1, and reduced NRF2 and Bcl-2 Associated X-protein respectively), and loss of neuron in the CNS, demonstrated using H&E, Golgi impregnation and CFV. Increased astrocytic densities with reactive astroglia were observed too. **Conclusion:** This finding revealed the neurobehavioral, structural and molecular alteration associated with SD+FEx exposure, although TCA was capable of offering protective remedy to the neurotoxic to neurodegenerative effects of SD+FEx exposure, making it a potential candidate for attenuating neurodegenerative diseases.

Disclosures: **J.I. Enya:** None. **M.A. Amadi:** None. **D.P. Finebone:** None. **S.C. Secundus:** None. **H.B. Akpan:** None. **S.O. Elijah:** None. **I. Onyeleonu:** None. **E.A. Esom:** None.

Poster

621. Neuroinflammation: Immunomodulators, Anti-Inflammatories

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 621.29

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: UVA Start-up

Title: Development of a novel model: Brain overgrowth without detrimental impacts on behavior or tissue integrity in a songbird

Authors: *E. A. SCALZI[1], T. A. LARSON[2];
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Abstract: Resources are limited in the brain, thus when one brain region expands, there is likely a cost in terms of limits on factors necessary for cell function and survival, but also in terms of physical space. The importance of resource allocation is even more important in brain regions undergoing dramatic, cyclical plasticity in neuronal number such as the song control nucleus, called HVC, in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelli*). Each breeding season, Gambel's white-crowned sparrows add around 60,000 neurons to an existing pool of around 100,000 neurons, doubling the total volume of HVC. Upon transition into nonbreeding conditions, an equal number of neurons die off each year. Lying immediately across the ventricle of HVC is the hippocampus, another brain region known for the addition of adult-born neurons. We hypothesized that hippocampus would grow just prior to the onset and after the end of breeding season to accommodate both the need of hippocampus for migratory behavior and for the conservation of space. Quantification of hippocampal volume and neuron number, however, revealed that plasticity of hippocampus paralleled that of HVC, suggesting that growth of the two regions causes an expansion of the total telencephalon volume. In mammals such expansion of brain volume is often detrimental, but can be ameliorated by decreases in cerebral spinal fluid. Thus, we are examining the plasticity of the vasculature, including the brain lymphatic vessels, to ask if the sparrow accommodates the growth of HVC and hippocampus through alterations in vascularization, lymphatic drainage, and intracranial pressure. Development of a model in which expansion of brain volume can be induced in a controlled fashion and in areas that regulate quantifiable behavior, would be extremely valuable for basic understanding of the mechanisms controlling morphology, pressure within cavities, and resource allocation. Uncovering mechanisms in the avian brain that allow for extreme growth without cost to other brain regions or behavior will lay the foundation for future development of therapeutics that could prevent or ameliorate pathological swelling in mammalian brains.

Disclosures: E.A. Scalzi: None. **T.A. Larson:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: An in vitro model of neuron degeneration for screening neuroprotective agents

Authors: *H. EL-BAROUDY, A. KRISHNAN, B. CHOWDHURY;
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Abstract: Peripheral nerve injuries often lead to loss of functions due to degeneration of distal axons and loss of functional connectivity. The extent of functional restoration depends on the degree of axon loss and the potential ability of neurons to regenerate. The neurons (neuronal soma) synthesize essential growth factors required for axon growth, especially during the initial phases of regeneration. However, whether the neurons that are disconnected from target tissues due to nerve damage stay healthy for the long term is unknown. While mixed reports exist, long-term studies indicated that sensory neurons enter apoptosis after severe distant axotomy. Hence, neuroprotective agents have high therapeutic value in nerve regeneration therapies. Studies exploring potential neuroprotectors have been active for some time. However, no *in vitro* model currently represents sensory neuronal apoptosis in response to nerve injury. Whole dorsal root ganglia (DRG) explant cultures are in place, but they represent a neurite extension model with no apoptosis induction in neurons. Developing an *in vitro* neuron apoptosis model may fast-track screening of novel neuroprotective agents. Here, we characterized an *in vitro* model representing spontaneous sensory neuron apoptosis in response to nerve injury. We used adult lumbar dorsal root ganglia (DRG)-nerve preparation to model the in vitro neuron degeneration. The DRG-nerve whole tissue preparation was embedded in the Cultrex extracellular matrix containing growth factors to mimic a 3D environment. Immunohistochemical analysis of this preparation was performed at different time intervals to characterize the onset of axon degeneration, neurofilament loss in neurons, and induction of neuronal apoptosis. We found satellite glial cell (SGC) wrapping around the injured neurons resembling the characteristic *in vivo* injury response in this *in vitro* preparation. TUNEL staining showed neuronal apoptosis, which was maximum at the 7-day interval. We found that early intervals in this model have intact axons and healthy neurons, enabling screening potential neuroprotectors. Overall, we developed a novel *in vitro* degeneration model, which can be used for screening nerve regeneration therapeutics, especially neuroprotectors.

Disclosures: H. El-Baroudy: None. **A. Krishnan:** None. **B. Chowdhury:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Circumvent NIH Grant 483614

Title: Novel therapeutic strategy for treating the lysosomal storage disease, infantile neuronal ceroid lipofuscinosis by administering proteinpalmitoyl thioesterase mimetic N-tert-butyl hydroxylamine

Authors: *Z. A. FYKE[1], A. YOSHII[2];
[1]Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL; [2]Anat. & Cell Biol., Univ. of Illinois, Chicago, IL

Abstract: Protein palmitoylation is a reversible mechanism that can regulate the subcellular localization, function and interactions of a wide range of proteins. Infantile neuronal ceroid lipofuscinosis (CLN1) is a devastating lysosomal storage disease in which mutations in protein palmitoyl-thioesterase *(PPT1),* leads to higher levels of palmitoylation in many synaptic proteins. The loss of function in the critical depalmitoylation enzyme PPT1, results in the accumulation and aggregation of overly palmitoylated proteins known as autofluorescent lysosomal storage material (ALSM), which drives microglial activation and reactive gliosis. CLN1 disease symptoms include blindness, seizures, and psychomotor dysfunction leading to premature death by 5 years of age. The disruption of palmitoylation proteostasis, microglial activation, and reactive gliosis are also signatures of a multitude of neurological diseases including Alzheimer's Disease, Huntington's Disease, and Schizophrenia. Therefore, the *Ppt1*$^{-/-}$ knock-out mouse strain may present implications beyond CLN1 disease and is being used to study the effect of supplementation with PPT1 mimetic, N-tert-butyl hydroxylamine (NtBuHA) . NtBuHA cleaves the thioester bonds that bind palmitate to cysteine residues, suggesting therapeutic replacement could potentially attenuate the deficits of *PPT1* $^{-/-}$ alongside other pathophysiolologically related neurological diseases. Consequently, we studied the effect of NtBuHA treatment on *Ppt1*$^{-/-}$ mouse primary neuronal cultures to examine the effect of enhanced depalmitoylation and found a significant decrease in ALSM. Subsequently, we examined the effect of NtBuHA treatment *in vivo*. Intriguingly, oral administration of NtBuHA to *Ppt1* $^{-/-}$ mice led to significant reductions in ALSM, GFAP, and IBA1 positive cells when compared to untreated knock-outs. Additionally, we began testing the effect of NtBuHA treatment on ameliorating ataxia, a characteristic of PPT1 deficiency. Preliminarily, treatment with NtBuHA improves motor coordination and endurance. These findings point to NtBuHA as a potential first clinical treatment for CLN1 disease and suggests the examination of compounds that target palmitoylation biology in related neurological pathologies.

Disclosures: **Z.A. Fyke:** A. Employment/Salary (full or part-time):; university of Illinois at Chicago. **A. Yoshii:** 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.; Circumvent Pharmaceudicals.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ramón y Cajal. RYC2020-029596-I
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Title: Cannabidiol ameliorates mitochondrial disease via the activation of PPARg

Authors: *E. PUIGHERMANAL[1], F. MENARDY[1], A. GELLA[1], G. VAN DER WALT[1], E. SANZ[2], A. QUINTANA[2];
[1]Neurosci. Inst., [2]Inst. of Neurosci. and Dept of Cell Biology, Physiol. and Immunol., Autonomous Univ. of Barcelona, Bellaterra, Spain

Abstract: Mitochondria are essential organelles that generate the majority of cellular ATP. Mutations in either nuclear or mitochondrial genomes can cause mitochondrial dysfunction and lead to a heterogeneous group of disorders known as primary mitochondrial diseases (MD), for which no treatments are approved. MDs are usually progressive, and often cause significant disability and premature death. Among them, Leigh syndrome (LS) is the most common pediatric MD and is generally characterized by neuromuscular affectation. Here, we show that daily cannabidiol (CBD) administration significantly extends lifespan and improves clinical complaints in two mouse models of LS. In particular, we found that CBD delays motor decline and the appearance of neurodegenerative signs, ameliorates social deficits, and decreases both the duration and intensity of thermally-induced seizures. These beneficial effects are correlated with decreased neuroinflammation in the globus pallidus, one of the main brain areas affected in LS. Moreover, we found that CBD's therapeutic effects require the activation of peroxisome protein activator receptor gamma (PPARg), a nuclear receptor involved in mitochondrial function, energy metabolism and inflammatory responses. Altogether, our study reveals a PPARg-dependent beneficial effect of CBD on a highly severe form of MD.

Disclosures: **E. Puighermanal:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **F. Menardy:** None. **A. Gella:** None. **G. Van der Walt:** None. **E. Sanz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **A. Quintana:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PAPIIT, UNAM, IN228420

Title: Prolactin neuroprotection against glutamate excitotoxicity is mediated via PI3K/AKT signaling pathway activation in primary culture rat hippocampal neurons

Authors: *G. MOLINA SALINAS, V. RODRIGUEZ-CHAVEZ, B. MARTÍNEZ NÁJERA, M. CERBON;
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Abstract: It has been well recognized that prolactin (PRL) is a pleiotropic hormone with multiplefunctions in different organs including the brain, where it participates in the induction of maternal behavior, neurogenesis, among others. Prominently, it has been reported that PRL has a significant role in neuroprotection against excitotoxicity damage, produced by glutamate (Glu) or kainic acid (KA) in both *in vitro* and *in vivo* models. However, the molecular mechanisms involved in PRL's neuroprotective effects in the hippocampus have not been completely elucidated. Therefore, the aim of the present study was to assess the signaling pathway by which PRL promotes neuronal survival in primary cultures of rat hippocampal neurons that were exposed to Glu excitotoxicity (Glu, 50 μM). These neurons were administrated with 20 ng/ml of PRL, PRL+Glu (20 ng/ml/ 50μM), Inhibitor LY294002 (50 μM/ml) and IPG (50 μM, 20 ng/ml/ 50μM). Here we show that phosphoinositide 3-kinases/Protein Kinase B (PI3K/AKT) signaling pathway activation is implicated in PRL effects against excitotoxicity damage promoting neuronal survival through upregulation of active AKT and its target proteins, after Glu administration. Inhibition of the PI3K/AKT signaling pathway abrogated the protective effect of PRL against glutamate-induced neuronal death. This result suggests a neuroprotective role for PRL mediated by AKT activation, largely implicated in neuroprotection. It is important to highlight the PRL signaling during neuroprotection in the brain as a target for future research to develop a possible treatment of neuronal damage found in many neurological and neurodegenerative diseases. This study received funding from PAPIIT, UNAM, IN228420.

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Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY028690
National Multiple Sclerosis Society

Title: Intranasally administered mesenchymal stem cell extracellular vesicles and optic neuritis

Authors: *D. OGBU[1], A. LUI[1], L. TORRES[1], D. L. FEINSTEIN[3], S. RAVINDRAN[2], S. ROTH[1];
[1]Anesthesiol., [2]Col. of Dent., Univ. of Illinois at Chicago, Chicago, IL; [3]Jesse Brown Veteran Affairs, Chicago, IL

Abstract: Optic neuritis (ON) is characterized by inflammation of the optic nerve which leads to degeneration of retinal ganglion cells (RGCs), death of RGC axons and ultimately vision loss in Multiple Sclerosis (MS) patients. Currently, there are no treatments that prevent loss of RGC axons in ON. Mesenchymal stem cells (MSCs) are neuroprotective for ON yet there are significant limitations, including aberrant growth and low survival rate in tissues. The paracrine effects of MSCs are mediated by extracellular vesicles (EV) which stimulate cellular responses once taken up by target cells. We administered intranasal native MSC EVs and engineered MSC EVs overexpressing anti-inflammatory microRNA in mice in a preclinical model of MS, Experimental Autoimmune Encephalomyelitis (EAE). Optical coherence tomography showed both types of EVs attenuated the loss of RGCs in EAE. The distribution of EVs has not been determined and the effect of EVs on retinal function is unknown. The aim of this study is to determine the localization of EVs in the optic nerve and brain after intranasal administration. We hypothesize that MSC EVs will localize to oligodendrocytes, axons, astrocytes, and microglia in the optic nerve and brain. In addition, this study aims to identify how EVs alter retinal function following intranasal treatment. We assessed the localization of intranasal MSC EVs in the optic nerve and brain after one day, three days, and seven days of administration via immunostaining. Oligodendrocytes, RGC axons, and astrocytes were stained for myelin basic protein, Beta-tublin3 and glial fibrillary acidic protein, respectively, microglia with IBA1, and neurons with NeuN. We measured retinal function using electroretinography for a- and b- waves, and scotopic threshold response for RGC function. MSC EVs were present in astrocytes and axons in the optic nerve, and in neurons in the olfactory bulb, cerebellum, and pons within 3 days after administration, persisting for at least 7 days after administration. These results suggest that MSC EVs may be an effective non-invasively administered treatment for vision loss in optic neuritis accompanying MS.

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Poster

622. Neuroprotection III

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Program #/Poster #: 622.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R35NS105076-01
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Adelson Medical Research Foundation

Title: Identification of target-specific therapeutics for the treatment of chemotherapy-induced peripheral neuropathy in human iPSC-derived neurons

Authors: *V. PETROVA[1], A. SNAVELY[1], C. E. MILLS[2], J. SPLAINE[3], J. A. SMITH[3], I. SINGEC[4], P. K. SORGER[2], C. J. WOOLF[1];
[1]Boston Children's Hosp., [2]Lab. of Systems Pharmacol., [3]ICCB-Longwood Screening Facility, Harvard Med. Sch., Boston, MA; [4]NCATS, NIH, Rockville, MD

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a common debilitating condition that arises from the toxicity of certain cancer chemotherapy agents to peripheral neurons and is characterized by sensory symptoms such as numbness, tingling, burning, and chronic pain. In a subset of patients, the severity of symptoms results in the termination of their anti-cancer treatment prematurely, which worsens their outcome. Currently, there are no approved drugs or measures to prevent or reverse CIPN, and the mechanisms leading to the nerve damage and pain are not understood. We recently discovered, a novel multi-kinase inhibitor, Compound X, as an effective neuroprotective agent in a large screen for neuroprotective compounds against vincristine-induced growth arrest in human iPSC-derived motor neurons. Compound X is also neuroprotective in a clinically relevant, *in vitro* model of paclitaxel-induced neurodegeneration in human iPSC-derived sensory neurons. To identify the downstream kinase targets of Compound X, we utilized the latter model as a high-throughput screening platform to screen a custom-made library of 192 kinase inhibitors that span the human kinome. 14 compounds were identified that reliably prevent or rescue neurodegeneration after paclitaxel exposure. It is imperative that any treatment developed for suppressing the side effects of paclitaxel on sensory neurons does not interfere with its ability to target tumor growth. Therefore, in a secondary assay, all neuroprotective compounds were screened for their effects on paclitaxel's anti-neoplastic properties in three human breast cancer cell lines. 2 of the hits suppressed paclitaxel’s toxicity in the cancer cell lines and were excluded from further analysis. The remaining hit compounds were then subjected to extensive computational, chemical, and pharmacological modeling and from this, we identified several kinases which could play a key role in chemotherapy-induced axon degeneration. The expression of these targets in the neurons was cross-validated with RNA sequencing data from our iPSC-derived human neurons and we are characterizing the targets using genetic ablation tools. Our multifaceted approach could result in both elucidating the mechanisms behind chemotherapy-induced peripheral neuropathy and a transition of novel therapeutics to the clinic which would improve many cancer patients' quality of life.

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Poster

622. Neuroprotection III

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Program #/Poster #: 622.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH AOD22011-001

Title: Adenosine A_1 Receptor (A_1AR) Agonist after Nerve Agent Intoxication is Life Promoting and Neuroprotective in a Humanized Esterase Mouse Soman Seizure Model

Authors: *C. MUNOZ, Z.-M. KEITH, C. ACON-CHEN, T.-M. SHIH;
United States Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: The anticipation of supportive human care during pre-clinical research is imperative for success, and simulating nerve agent (NA) intoxication akin to humans is vital to test novel countermeasure efficacy. The A_1AR agonist N-bicyclo-(2.2.1)hept-2-yl-5'-chloro-5'-deoxyadenosine (ENBA) was shown to be a potent anticonvulsant and neuroprotective (A/N) compound in rat seizure models using soman (GD) and sarin (GB). An advanced investigation of the A/N efficacy of ENBA was conducted using a human acetylcholinesterase (AChE) gene knock-in (KI) and mouse serum carboxylesterase gene knock-out (KO) mouse (C57BL/6-Ces1c$^{tm1.1Loc}$AChE$^{tm1.1Loc}$/J), identified here as "KIKO," to mimic human AChE-specific treatment response. Here, we investigated delayed treatment of ENBA against standard NA medical countermeasures (atropine sulfate plus 2-PAM and midazolam), and in combination, to directly assess ENBA for potential as a countermeasure against GD. Specifically, male KIKO mice implanted with cortical electroencephalographic (EEG) electrodes were pretreated with the oxime HI-6 30 minutes prior to challenge with GD (33 µg/kg, SC). ENBA was given at 15 mg/kg (IP), the minimum dose with 100% suppression of sustained *status epileptics* (SSE), after 15 minutes of ongoing SSE activity (relevant to a civilian emergency triage). To assess the latency of recovery a 14-day endpoint was chosen. We collected and compared EEG, seizure suppression outcomes, temperature, toxic signs, lethality and neuropathology. Without ENBA, succumbing to death from GD exposure was high (45%) at 14 days. When ENBA was included in the treatment (alone or in combination) lethality decreased to 15%. Additionally, SSE was suppressed quickly, neuropathology was absent, and animals reached recovery by 48 hours after GD exposure. Thus, we have replicated our previous results of powerful A/N offered from adjunct ENBA after NA exposure in rats in humanized KIKO mice. ENBA intervention sustained seizure suppression and aided neurorecovery when in combination with transient post-treatment supportive care. Furthermore, this mouse model promotes healthy reversal of the residual thermo-depressive and cardiovascular effects that full adenosine receptor agonism produces. Thus, ENBA has a compelling case for potential use in delayed therapy to victims of NA exposure and supports consideration of the development for human trial.

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Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2R25GM060507
P20MD006988

Title: Dietary DHA-rich supplementation decreases neurotoxic lipid mediators in participants with Type II Diabetes and Neuropathic Pain

Authors: *C. YOO, A. M. DURAN, L. SALTO, W. BEESON, A. FIREK, F. ALMAGUEL, M. DELEÓN;
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Abstract: Diabetic neuropathy (DN), also known as distal symmetrical polyneuropathy, affects up to 50% of patients with type 2 diabetes and is a significant cause of morbidity and increased mortality. Of those patients experiencing DN symptoms, about half report neuropathic pain. Chronic hyperglycemia has been central to the pathogenesis of painful diabetic neuropathy (pDN). However, emerging evidence suggests that dyslipidemia and dysfunctional lipid metabolism may be the underlying reason for the onset of pDN. More specifically, the accumulation of neurotoxic lipid derivatives, such as palmitic acid and stearic acid derivatives, are demonstrated in the literature to cause neuroinflammation and mitochondrial dysregulation. Recently, we reported that a docosahexaenoic acid (DHA)-enriched dietary supplementation improved neuropathic pain symptoms in patients with type 2 diabetes. Furthermore, after untargeted metabolomics analysis of patient plasma samples, before and after dietary intervention, circulating plasma metabolites showed a significant shift toward decreased reactive oxygen species biosynthesis, lipid peroxidation, improved Ca2+ homeostasis, and increased glutathione activity. In this follow-up study, we sought to understand the impact of enriched DHA supplementation on the patients' plasma lipidome. Our approach used a quantitative untargeted lipidomic analysis, allowing for the unbiased interpretation of the lipid-mediators involved with pDN and determining the impact of the dietary omega-3 PUFA supplementation on the resolution of neurotoxic lipids. From the comprehensive lipidomic dataset, data were extracted and then analyzed by random forest, hierarchical clustering, ingenuity pathway analysis, and metabolic pathway mapping to pinpoint the role of DHA in altering the lipid profiles of our pDN patients. The results indicate that neurotoxic fatty acid derivatives implicated in increasing neuroinflammation and neuronal injury were decreased, whereas DHA derivatives involved in ameliorating pDN symptoms were increased. For example, we measured significant decreases in C16:0 and C18:0 derivatives, shown to elicit neuropathic pain, without change in total fatty acids C16:0 or C18:0 concentrations. Also, a dramatic shift from docosatetraenoic acid (22:4) to docosahexaenoic acid (22:6) fatty acid derivates involved with cell membrane turnover was measured. This study demonstrates that dietary DHA supplementation may be a complementary strategy to reduce adverse symptoms associated with neuroinflammatory diseases and painful neuropathy.

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Poster

622. Neuroprotection III

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Program #/Poster #: 622.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Saint Louis University Graduate-undergraduate collaboration Fund
Saint Louis University Presidential Research Fund

Title: Effect of ERK1/2 and GSK3β-mediated signaling on progranulin's neuroprotection against hyperglycemic stress

Authors: *C. DEDERT[1], F. XU[2];
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Abstract: Hyperglycemia is a defining characteristic of type II diabetes and contributes to neurodegenerative disease through buildup of glycated proteins and dysregulation of autophagy, a cellular protein degradation pathway. There is evidence that progranulin (PGRN), a neurotrophic factor implicated in protection against frontotemporal lobar dementia, may protect against hyperglycemia-induced neuronal damage through regulation of autophagy flux. In previous studies, we have observed changes in phosphorylation of ERK1/2 and GSK3β in response to PGRN under high-glucose stress, and sought to confirm the role of these kinases in PGRN's mechanism of action. Primary cortical cells from E18 Sprague-Dawley rat pups were cultured to maturity before incubation in high glucose medium with PGRN and pharmacological inhibitors of ERK1/2 and GSK3β. Cell viability was observed using fluorescence-based reporter assays and phase-contrast microscopy. Autophagy function was assessed through western blot, immunofluorescence, and protein turnover assays. Our results showed that acute (72 hour) high-glucose treatment reduced cellular viability and increased neuritic beading, which were improved by PGRN and reversed when ERK1/2 and GSK3β were inhibited. Impairment of autophagy, measured through western blot and immunofluorescence monitoring of the autophagosome marker LC3B and lysosome marker LAMP2A, were also altered by PGRN in a manner dependent on ERK1/2 and GSK3β. Lastly, protein turnover, determined by fluorescence of the protein substrate DQ-BSA, decreased under high glucose, but was preserved by PGRN in an ERK1/2 and GSK3β-dependent manner. These findings indicate that progranulin's mechanisms of neuroprotection and autophagy regulation under hyperglycemic stress are mediated by ERK1/2 and GSK3β signaling. However, more work needs to be performed on *in vivo* and gene knockout models to verify these findings.

Disclosures: **C. Dedert:** None. **F. Xu:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-NINDS Grant R01 NS113921
NIH-NINDS Grant R01 NS107417

Title: Cocktail systemic treatment with pyrazolone and chlorpromazine can activate the proteasome in neonatal pig brain

Authors: *A. AMREIN ALMIRA[1], C. JAVDAN[1], J. K. LEE[1], L. J. MARTIN[2];
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Abstract: Current treatments for neonatal hypoxic-ischemic encephalopathy (HIE) are not injury mechanism-targeted. Proteasome dysfunction and proteinopathy are understudied mechanisms of neurodegeneration in HIE. We seek to identify small molecule activators of the brain proteasome in a clinically relevant neonatal piglet model to propose mechanism-specific therapies. Neonatal piglets (1.5 - 2.0 kg, male) were sedated, intubated, and anesthetized for physiological monitoring and intravenously (iv) received a cocktail of pyrazolone (PYR, 10 mg/kg) and chlorpromazine (CPZ, 1 mg/kg) (N = 4). PYR and CPZ were identified by others to be activators of the 20S proteasome in cell culture-based small molecule screens and in a mouse model of neurodegeneration. Control piglets received vehicle (DMSO/ethanol/saline, N = 4). Piglet hemodynamics were monitored for 3 hours after drug/vehicle delivery, after which piglets were recovered from anesthesia and survived. Average mean arterial pressure was 65 ± 4 mmHg in drug-treated piglets and 72 ± 6 mmHg in vehicle. Average heartrate was 166 ± 18 BPM in drug-treated piglets and 198 ± 25 BPM in vehicle. A second dose of PYR/CPZ or vehicle was delivered iv 12 hours after the first dose. 24 hours after the initial dosing, the piglets were exsanguinated with ice-cold phosphate-buffered saline perfusion. The frontal cortex (FC), cerebellum (CB), subcortical white matter (WM), and caudate nucleus (CN) were precisely microdissected, homogenized, and combined with chymotrypsin-like proteasome activity substrate SUC-LLVY-AMC in duplicate in a black flat-bottom 96-well pate. To specifically isolate fluorescent signal produced by AMC liberation due to proteasome activity, MG-132 was used as an inhibitor in separate control wells. Fluorescence was immediately measured with 360nm excitation/460nm emission filters at 37°C over 15 minutes. Across all treatments and brain regions, proteasome activity was detected as an increase in fluorescence units over time. Total AMC fluorescence was reduced 65 - 85% by MG-132. In FC, CB, and WM, 50% (N=2) of treated piglets saw a steeper rate of increase in fluorescence over time compared to vehicle-treated piglets. Other piglets did not respond (1 of 4) or had a decreased rate (1 of 4). In CN (N=2), there was no observed increase in activity. No difference in activity was observed among different brain regions. The chymotrypsin-like activity of the proteasome in neonate piglet FC,

CB, and subcortical WM can respond to intravenous treatment of PYR and CPZ in combination without apparent untoward off-target physiological actions. Studies in larger cohorts are needed to further characterize this response.

Disclosures: A. Amrein Almira: None. **C. Javdan:** None. **J.K. Lee:** F. Consulting Fees (e.g., advisory boards); United States Food and Drug Administration, Edwards Life Sciences. **L.J. Martin:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Targeting an allosteric site in dynamin-related protein 1 to inhibit Fis1-mediated mitochondrial dysfunction

Authors: L. RIOS, ***S. POKHREL**, B. HAILESELASSIE, D. MOCHLY-ROSEN; Stanford Univ., Stanford, CA

Abstract: The large cytosolic GTPase, dynamin-related protein 1 (Drp1), localizes to mitochondria via adapter proteins and mediates mitochondrial fission. Cell stress triggers Drp1 binding to the mitochondrial adapter Fis1, causing pathological mitochondrial fission involving mitochondrial fragmentation, ROS production, metabolic collapse, and cell death. Selective disruption of the Drp1-Fis1 interaction can selectively inhibit pathological mitochondrial fission without affecting physiological mitochondrial fission mediated by Drp1 binding to mitochondrial fission factor (Mff) on the mitochondrial surface. A peptide-inhibitor of the Drp1-Fis1 interaction, P110, reduces pathology in numerous models of neurodegeneration, ischemia, and sepsis, without blocking physiological functions of Drp1. P110 treatment ameliorates motor function and survival in amyotrophic lateral sclerosis and reduces amyloid plaques and cognitive decline in Alzheimer's disease mouse models. Because peptide therapeutics have pharmacokinetic limitations, we searched for small molecules that mimic P110’s benefit. We first mapped the P110 -binding site to a switch I-adjacent grove (SWAG) on Drp1. Screening for SWAG-binding small molecules identified SC9, and SC9 selectively disrupts Drp1-Fis1 interaction and prevents pathological mitochondrial fission in cells and reduces inflammatory factors and improves survival in a mouse model of sepsis. Because of this, SC9 and blood brain barrier penetrant SC9 analogs may be potential therapeutics for neurodegenerative disease indications.

Disclosures: L. Rios: None. **S. Pokhrel:** None. **B. Haileselassie:** None. **D. Mochly-Rosen:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: AAV-mediated AIBP delivery protects retinal ganglion cells in glaucomatous neurodegeneration

Authors: *S. CHOI[1], W.-K. JU[2], K.-Y. KIM[3], G. PERKINS[3], J. KIM[1], Y. MILLER[1]; [1]Med., UCSD, La Jolla, CA; [2]Ophthalmol, Univ. California San Diego, La Jolla, CA; [3]Natl. Ctr. Microscopy and Imaging, Univ. of California San Diego, La Jolla, CA

Abstract: AAV-mediated AIBP delivery protects retinal ganglion cells in glaucomatous neurodegeneration
Won-Kyu Ju[1], Keun-Young Kim[2], Guy A. Perkins[2], Jungsu Kim[3], Yury I. Miller[3], Soo-Ho Choi[31]Hamilton Glaucoma Center and Shiley Eye Institute, The Viterbi Family Department of Ophthalmology, University of California San Diego, La Jolla, CA 92039, USA. [2]National Center for Microscopy and Imaging Research, Department of Neurosciences, University of California San Diego, La Jolla, CA 92039, USA. [3]Department of Medicine, University of California San Diego, La Jolla, CA 92039, USA.
Glaucoma is a leading cause of irreversible blindness worldwide and characterized by a slow, progressive, and irreversible degeneration of retinal ganglion cells (RGCs) and their axons, resulting in loss of visual function. Glia-driven neuroinflammation and mitochondrial dysfunction play critical roles in glaucomatous neurodegeneration. In previous study, we demonstrated that apolipoprotein A-I binding protein (AIBP; gene name *APOA1BP* or *NAXE*) deficiency induces upregulation of toll-like receptor-4 (TLR4) and interleukin-1β (IL-1β) as well as impairment of mitochondrial network and function and oxidative stress in the retina, resulting in RGC death and vision dysfunction. In the current study, we found that the expression of AIBP and ATP-cassette transporter 1 (ABCA1) were downregulated while TLR4 and IL-1β expression were increased in glaucomatous human and mouse retinas. In glaucomatous DBA/2J mice, overexpression of AIBP delivered by AAV promoted RGC survival, reduced cholesterol deposition, inhibited TLR4 and IL-1β activation, and preserved retina and brain connectivity. Administration of recombinant AIBP protein reduced mitochondrial dysfunction and TLR4/IL-1β activation in Müller glial cells exposed to elevated hydrostatic pressure. These findings support the notion that AAV-AIBP has the therapeutic potential to protect RGCs and their axons via inhibiting glial-driven neuroinflammation in glaucoma.
Conflict of interest : S.H.Choi, W.K.Ju, and Y.I. Miller are co-inventors listed in patent applications related to the topic of this study. Y.I.M is scientific co-founder of Raft Pharmaceuticals LLC. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies. Other authors declare that they have no competing interests.

Disclosures: **S. Choi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventors in

patent applications related to the topic of this study. **W. Ju:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventors in patent applications related to the topic of this study. **K. Kim:** None. **G. Perkins:** None. **J. Kim:** None. **Y. Miller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventors in patent applications related to the topic of this study and scientific co-founder of Raft Pharmaceuticlas LLC..

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NEI Grant R01EY032542
NIH/NEI Grant R21EY033060
The Buoniconti Fund

Title: In vivo identification of long intergenic non-coding RNAs involved in regulatingneuronal survival after axonal injury

Authors: *G. NASCIMENTO DOS SANTOS[1], K. LEVAY[1], A. C. AYUPE[1], J. K. LEE[1], K. SATKUNENDRARAJAH[2], K. K. PARK[1];
[1]Dept. of Neurolog. Surgery, Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Long projection neurons, including retinal ganglion cells (RGCs) degenerate after axonal injury. Much effort has been made to identify effective therapeutic approaches to rescue these compromised neurons. We recently performed a systematic analysis on RNA-sequencing data and identified long non-coding RNAs (lncRNAs) whose expressions are differentially up-regulated in distinct subtypes of RGCs after axonal injury. Herein we examine the neuroprotective effects of lncRNAs using adeno-associated virus (AAV)-mediated gene knockdown and optic nerve crush (ONC) in mice. Adult mice received unilateral intravitreal injection of shRNA against lncRNAs whose expression are robustly increased after optic nerve crush. Since many of these lncRNAs are unannotated, here we name these lncRNAs *optic nerve induced lncRNA (i.e., Onil1 and Onil2).* Two weeks after ONC, RGC survival was determined using an antibody against RBPMS, a marker for RGCs. AAV-mediated expression of lncRNA was performed to determine if these lncRNAs affect RGC survival when ectopically expressed. Retinal sections were stained for ATF3 to examine if lncRNAs modulate early stress response. Expression of lncRNAs were validated using BaseScope in situ hybridization (ISH). Two weeks after ONC, RGC survival was markedly higher in the shRNA-*Onil1* compared to the shRNA-scramble control. In contrast, *Onil1* overexpression did not change RGC number after ONC or in the uninjured retina. ISH revealed strong induction of *Onil1* transcripts in RGCs 3 days after

injury. Two weeks after ONC, shRNA-*Onil1* group displayed a lower number of $ATF3^+$ RGCs than the control group, suggesting that *Onil1* knockdown affects the early stress responses. To examine if *Onil1* induction occurs in other CNS systems, we examined *Onil1* expression in mouse spinal cords. Strikingly, we find increase in *Onil1* expression in subsets of cells in the spinal cords after different types of spinal cord injury, suggesting that *Onil1* is involved in the death of different types of neurons. Lastly, to investigate the potential mechanisms underlying the *Onil1* knockdown effect, we performed Comprehensive identification of RNA-binding proteins by mass spectrometry to identify *Onil1* binding proteins. We and others show that the majority of lncRNAs are expressed in a highly tissue and cell-type specific manner, making them potential highly efficacious targets for therapeutic treatment. Our data show that distinct lncRNAs are induced after injury, and that one lncRNA regulates cell death. Defining *Onil1*'s molecular targets and its underlying mechanisms may lead to the development of a therapeutic intervention to prevent neuronal loss after injury.

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Poster

622. Neuroprotection III

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Program #/Poster #: 622.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
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the Medical Technology Enterprise Consortium
UCSF Program for Breakthrough Biomedical Research

Title: The atypical receptor-ligand pair Ocm-R/oncomodulin enables axon regeneration in the central and peripheral nervous systems

Authors: *L. XIE[1], Y. YIN[2], S. L. PETERSON[3], S. S. JAYAKAR[4], C. SHI[5], B. LENFERSTURNES[6], Z. ZHANG[7], J. OSES-PRIETO[8], J. LI[9], A. BURLINGAME[8], C. J. WOOLF[10], M. RASBAND[11], L. I. BENOWITZ[12];
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MA; [11]Baylor Col. of Med., Houston, TX; [12]Labs Neurosci Res. Neurosurg, Boston Children's Hosp/Harvard Med. Sc, Boston, MA

Abstract: The atypical receptor-ligand pair Ocm-R/oncomodulin enables axon regeneration in the central and peripheral nervous systems

Lili Xie[1,2], Yuqin Yin[1,2], Sheri Peterson[1,2], Selwyn Jayakar[2,3], Caleb Shi[4], Bruna Lenfers Turnes[2,3], Zihe Zhang[2,3], Juan Oses-Prieto[5], Jian Li[6], Al Burlingame[5], Clifford J. Woolf[2,3], Matthew Rasband[7], and Larry I. Benowitz[1,2,8*]
[1]Department of Neurosurgery, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115. [2]F.M. Kirby Neurobiology Center, Boston Children's Hospital, Boston, MA 02115. [3]Department of Neurology, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115. [4]Harvard College, Cambridge, MA 02138. [5]School of Pharmacy, University of California San Francisco. [6]Division of Rheumatology, Inflammation and Immunity, Brigham and Women's Hospital and Department of Medicine, Harvard Medical School, Boston, MA 02115. [7]Baylor College of Medicine, Houston TX. [8]Department of Ophthalmology, Harvard Medical School, Boston, MA 02115.

AbstractIntraocular inflammation enables retinal ganglion cells (RGCs) to regenerate injured axons through the optic nerve, an effect that is mediated in large part by the small Ca^{2+}-binding protein oncomodulin (Ocm) (Yin et al., 2006, 2009). Ocm also mediates the accelerated axon regeneration through peripheral nerves (PNs) (Niemi et al, 2022) that occurs after a conditioning PN lesion. Using a modified proximity biotinylation strategy, co-immunoprecipitation, surface plasmon resonance, and ectopic expression, we identify a protein tentatively named Ocm-R as a high-affinity Ocm receptor that mediates these phenomena. Deletion of this highly atypical growth factor receptor in RGCs suppresses inflammation-induced optic nerve regeneration, and deletion in peripheral sensory neurons suppresses the effects of a conditioning lesion in accelerating PN regeneration as well as the regeneration of dorsal column axons after spinal cord injury. Conversely, Ocm (with appropriate co-factors) strongly accelerates peripheral nerve regeneration. Thus, Ocm acting through Ocm-R enables axon regeneration in both the CNS and PNS.

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Poster

622. Neuroprotection III

Location: SDCC Halls B-H

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Program #/Poster #: 622.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 AA025967
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NIH P50 AA022534

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Title: The NLRP3 inflammasome plays a key role in neuropathic pain susceptibility in prenatal alcohol exposed (PAE) mice.

Authors: *M. S. SUN[1], S. NOOR[1], C. D. RUFFANER-HANSON[1], J. E. SANCHEZ[1], M. V. NYSUS[2], A. A. PASMAY[1], D. C. JIMENEZ[1], C. F. VALENZUELA[1], E. D. MILLIGAN[1]; [1]Dept. of Neurosciences, [2]Comprehensive Cancer Ctr., Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM

Abstract: Aberrant neuroimmune interactions often result in chronic pathological pain states. Allodynia, which is pathological sensitivity to non-painful light touch, is a common clinical pain problem. Our prior work demonstrated in rodent models that prenatal alcohol exposure (PAE) is a risk factor for developing allodynia from minor sciatic nerve injury in adult offspring. The observed allodynia in PAE offspring co-occurs with potent elevation of spinal and perineural proinflammatory cytokines including interleukin (IL)-1β. The NLRP3 inflammasome protein complex is made up of several intracellular and interactive signaling proteins, often activated by injury- or damage-derived factors. Once activated, the NLRP3 inflammasome converts precursor pro-IL-1β protein into active and releasable IL-1β. Here, we hypothesized that enhanced activation of the NLRP3 inflammasome plays a key role underlying neuropathic pain susceptibility from PAE. To accomplish this, a modification of the widely used sciatic nerve chronic constriction injury (CCI) mouse model was used to create minor sciatic injury with the goal to unmask the allodynia susceptibility caused by PAE. Additionally, we sought to determine whether blocking NLRP3 inflammasome action via a selective inhibitor, MCC950, could reverse full-blown allodynia in typical C57BL/6 unexposed mice with the standard CCI injury, or in PAE mice with minor CCI. For PAE, C57BL/6 mouse dams were provided saccharin (Sac)-sweetened alcohol (5%) or Sac-sweetened water for 4 hours/day during gestation (blood ethanol concentrations reach ~60 mg/dl) considered moderate alcohol exposure. Adult PAE and Sac offspring (~4 mo.) underwent minor (single sterile chromic gut 6-0 suture) CCI. Unexposed mice (~4 mo.) underwent standard CCI (three 5-0 chromic gut sutures). Prior to and after CCI, hindpaw sensitivity to light touch stimuli using calibrated monofilaments was assessed in male and female mice at designated timepoints throughout a 34-day and 20-day timecourse. Following established allodynia (Day 14 post-surgery), mice were given intravenous (i.v.) MCC950, or vehicle, followed by re-assessment of hindpaw responses. Data demonstrate that PAE mice but not Sac controls develop unilateral allodynia following minor injury. Blocking NLRP3 activation with i.v. MCC950 reversed allodynia in all mice treated with CCI, with maximal effects observed by 90 minutes and persisted for 3 days. These data suggest that the NLRP3 pathway (1) is sensitized in adult mice with PAE that may underlie PAE-induced allodynia susceptibility, (2) may be a target for novel treatment approaches to control allodynia following peripheral nerve injuries.

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Poster

622. Neuroprotection III

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Program #/Poster #: 622.16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: BHRI at Kent State University Pilot Award

Title: The methyl donor betaine is neuroprotective in EAE

Authors: K. KNIES[1], M. W. PSENICKA[3], J. L. WILLIAMS[3], *J. MCDONOUGH[2]; [2]Biol. Sci., [1]Kent State Univ., Kent, OH; [3]Neurosci., Lerner Res. Institute, Cleveland Clin., Cleveland, OH

Abstract: Multiple sclerosis (MS) causes great disability with few treatment options. Most of the disease modifying therapies for MS suppress the immune system, however, there are no effective therapies to stop the neurodegeneration which occurs and neuroprotective therapies are desperately needed. It has been established that damage to mitochondria contributes to neurodegeneration in MS. In addition to an imbalance in energy metabolism, other metabolic changes have also been reported in MS. We have previously shown that one carbon metabolism is dysregulated in MS. Most notably, the methyl donor betaine, also known as trimethylglycine, is depleted in the MS brain. We have also shown that this loss of betaine in MS is linked to a dysfunction of neuronal mitochondria. In the present study, we injected betaine intraperitoneally to treat disability in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. To induce EAE, mice were immunized with a myelin oligodendrocyte glycoprotein peptide and adjuvants and clinical motor disability was assessed. Then betaine administration began at the peak of disease disability (about 15 days post-immunization). We found a significant improvement in motor ability in betaine treated mice compared to EAE mice given vehicle during the chronic stages of disease (day 25-40 post-immunization). We then evaluated NeuN+ neurons by fluorescent immunohistochemistry in the ventral horn of the lumbar spinal cord and found that, as expected, the number of NeuN+ cells were significantly decreased in EAE mice compared to control mice (Control 281 +/- 90; EAE 168 +/- 23). In EAE mice given betaine, numbers of NeuN+ cells were significantly improved compared to EAE mice given vehicle (EAE + vehicle 168 +/- 23; EAE + betaine 238 +/- 36). We also observed mitochondrial functioning by evaluating SMI32, a marker for axons, and COX5B, a subunit of the cytochrome c oxidase complex, at the ventral horn of the lumbar spinal cord through fluorescent immunohistochemistry and colocalization. Our data show that COX5B staining was increased inside SMI32+ axons in EAE mice administered betaine compared to EAE mice given vehicle (n = 3 mice/group). These data indicate that betaine can help alleviate motor disability and improve mitochondrial functioning and reduce neuronal cell death in the EAE model of MS. Statistical analyses were done with either a one-way ANOVA or student's T-test with $p < 0.05$ considered significant.

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Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DARPA grant 140D0419C0092

Title: Stimulation of the spleen with electrical pulses or with ultrasound energy from a portable therapeutic device decreases cytokine release in acute inflammation rat model

Authors: *C. KAISER[1], D. P. ZACHS[2], A. TUMA[4], M. NEWHOFF[2], H. H. LIM[3];
[1]Claire Kaiser, [2]Univ. of Minnesota, [3]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN;
[4]Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Peripheral focused ultrasound stimulation (pFUS) of abdominal nerves and organs is a promising tool for non-invasive modulation of the autonomic nervous system. Recently published research indicates that pFUS can alter signaling pathways associated with inflammation control when focused near the spleen and glucose homeostasis when focused near the liver with implication for treatment of rheumatoid arthritis and sepsis, or hyperglycemia and obesity, respectively. Our group has previously shown that pFUS directed at the spleen decreases joint inflammation in a mouse model of arthritis, presumably by activating the cholinergic anti-inflammatory pathway (CAP). In CAP, efferent vagus and splenic nerve activity leads to release of norepinephrine in the spleen, triggering a signaling cascade involving CD4 T-cells and macrophages that leads to inhibition of pro-inflammatory cytokine release. CAP activation by invasive electrical stimulation of the cervical vagus nerve in animal models decreased pro-inflammatory cytokines such as TNF-alpha, IL-1beta, and IL-6. Electrical stimulation of the splenic neurovascular bundle (SNS) may garner similar results while avoiding modulation of off-target autonomic systems.
Our group is investigating the mechanism of action and optimal parameters for splenic pFUS (spFUS) compared to SNS. We are also evaluating a portable therapeutic ultrasound delivery device capable of precision targeting of the human spleen and modified for animal use. We assessed efficacy of spFUS and SNS for treatment of acute inflammation in anesthetized rats injected with lipopolysaccharides, a model of sepsis. Cytokine levels were assessed within circulating blood every 30 minutes and within the spleen at termination to isolate the time-dependencies in the cytokine cascade. Immunohistochemistry was performed to characterize splenic nerve location and size within the neurovascular bundle using neurofilament and tyrosine hydroxylase antibodies. Electrophysiology was performed to characterize the level of peripheral nerve activation by each stimulation modality. SNS significantly decreased serum levels of TNF-alpha and IL-1beta, as well as trends in GRO and IL-10. Stimulation with spFUS from the portable ultrasound device targeting large neurovascular bundles entering the spleen also showed consistent reductions in inflammatory cytokines with several differing time-dependent trends

across cytokines. This research further supports the potential of spFUS for driving anti-inflammatory responses in line with electrical stimulation approaches.

Disclosures: **C. Kaiser:** A. Employment/Salary (full or part-time):; SecondWave Systems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SecondWave Systems. **D.P. Zachs:** A. Employment/Salary (full or part-time):; SecondWave Systems. **A. Tuma:** None. **M. Newhoff:** None. **H.H. Lim:** A. Employment/Salary (full or part-time):; SecondWave Systems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SecondWave Systems.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Neurological Foundation of NZ
MBIE Smart Ideas
Health Research Council of NZ

Title: Kappa opioid receptor agonists promote oligodendrocyte maturation, remyelination and functional recovery in preclinical mouse models

Authors: R. BIBI[1], K. PATON[1], A. BIGGERSTAFF[1], L. DENNY[1], K. ROBICHON[1], D. LUO[3], A. LA FLAMME[2], T. E. PRISINZANO[3], ***B. KIVELL**[1];
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Abstract: In multiple sclerosis (MS) the body's own immune system attacks and destroys the protective myelin sheath surrounding axons leading to impaired saltatory conduction and a range of symptoms such as muscle weakness, fatigue, impaired motor function and paralysis. There is no cure for MS and current treatments target the immune system, however, none initiate myelin repair and enable functional recovery. In MS the failure of oligodendrocyte precursor cells (OPCs) to differentiate into mature myelinating oligodendrocytes is reported to be responsible for lack of repair leading to progressive functional decline. Activation of the kappa opioid receptor (KOR) has recently been shown to promote OPC differentiation onto mature oligodendrocytes (OL). In this study utilising primary cultures of mouse OPCs and high-throughput confocal microscopy techniques, we compareed the ability of KOR agonists to promote OPC differentiation into mature oligodendrocytes *in vitro*. Utilising two preclinical models of demyelination in C57BL/6J mice, we also showed KOR agonists promoted remyelination and functional recovery. In the experimental autoimmune encephalomyelitis

(EAE) model, we found that nalfurafine and salvinorin A analogues, β-tetrahydropyran salvinorin B, ethoxymethyl ether salvinorin B, and Mesyl Sal B, effectively decreased disease severity (paralysis) in a KOR-dependent manner and led to a greater percentage recovery compared to the traditional KOR agonist U50,488. In the cuprizone-induced demyelination model, we showed that the KOR agonists nalfurafine and ethoxymethyl ether salvinorin B led to an increase in the number of mature oligodendrocytes, the number of myelinated axons and the myelin thickness in the corpus callosum. This provides evidence that KOR agonists are a promising target for the development of pharmacotherapies targeting repair and remyelination.

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Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Conacyt 1021472

Title: Antinociceptive effect of clavulanic acid in paclitaxel-induced neuropathic pain in mice

Authors: *L. BALCÁZAR OCHOA[1], R. VENTURA-MARTÍNEZ[1], G. ÁNGELES LÓPEZ[1], E. ALVAREZ[2], P. AGUILERA[3];
[1]Natl. Autonoma Univ. Mexico, Mexico city, Mexico; [2]Neurociencias, Inst. Nacional De Psiquiatria, Mexico City, Mexico; [3]Inst. Nacional de Neurologia y Neurocirugia, Mexico Distrito Federal, Mexico

Abstract: ANTINOCICEPTIVE EFFECT OF CLAVULANIC ACID IN PACLITAXEL-INDUCED NEUROPATHIC PAIN IN MICE.LG BALCÁZAR-OCHOA1, R VENTURA-MARTÍNEZ1, GE ÁNGELES-LÓPEZ1, E ÁLVAREZ-SALAS3, PAGUILERA21Departamnto de Farmacología. Facultad de Medicina. Universidad Nacional Autónoma de México,CdMx, México.2Laboratorio de Patología Vascular Cerebral, Instituto Nacional de Neurología y Neurocirugía ManuelVelasco Suárez, CDMX, México3Molecular Neurophysiology, Department of Neuroscience Research, National Institute of Psychiatry,Calzada México-Xochimilco 101, Tlalpan, 14370 México, D.F., MexicoAbstract:Clavulanic acid (CLAV), a beta lactam molecule without intrinsic antibiotic

activity, has antinociceptiveeffect in inflammatory and neuropathic pain models in rodents. Paclitaxel (PCX), a frequently usedantineoplastic drug, produces difficult-to-treat neuropathic pain that is dose-limiting. The objective of thisstudy was to evaluate the antinociceptive effect of CLAV in PCX-induced neuropathic pain (PINP) in mice.PINP was induced in mice by intraperitoneal administration of 2 mg/kg of PCX every third day for sevendays to reach a cumulative dose of 8 mg/kg. The mechanical hyperalgesia PCX-induced was evaluatedusing the Von Frey filaments while cold allodynia was evaluated with the acetone paradigm. Once micedeveloped allodynia, CLAV was administered at different doses and its antiallodynic and antihyperalgesiceffects were evaluated. The area under the curve (AUC) analysis in the mechanical allodynia showed thatthe CLA (31.6 and 100 mg/kg) induce an antiallodynic effect significantly different with the vehicle in micewith PINP (56.89±13.21 and 104.4±18.19 vs 4.54± 1.26 au, respectively). While, in the acetone test, CLAV(3.1, 10, 31.6 and 100 mg/kg) also induce an antihyperalgesic effect significantly different to the vehiclegroup (143.9±17.36, 140.6±13.5, 174.5± 22.45 and 211.3±27.34 vs 94.6± 15.33 au, respectively). Inconclusion our results shows that CLAV, administered as a single dose, induced antihyperalgesic andantiallodynic effects in PINP mice. Since CLAV is already approved for clinical use, it represents a feasiblealternative for the treatment of neuropathic pain, improving the quality of life of patients under PCXtreatment.DisclosuresLG Balcázar-Ochoa: None. R. Ventura-Martinez: None. GE Angeles-López: None.Keywords: Clavulanic acid; neuropathic pain; paclitaxel; antiallodynic effect; antihyperalgesic effect.

Disclosures: L. Balcázar Ochoa: None. **R. Ventura-Martínez:** None. **G. Ángeles López:** None. **E. Alvarez:** None. **P. Aguilera:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NINDS R03NS121831
RTI IRD fund

Title: Identification and characterization of novel small molecule antagonists of GPR17 receptor

Authors: *S. NARAYANAN[1], A. M. DECKER[1], E. C. TONETTI[1], V. VASUKUTTAN[1], T. L. LANGSTON[1], S. P. RUNYON[1], X. LIU[2], R. Q. LU[2];
[1]RTI Intl., Research Triangle Park, NC; [2]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: GPR17 is a class A, G protein-coupled receptor, predominantly expressed in oligodendrocyte precursor cells (OPC) in the central nervous system. Studies with GPR17 knockout and overexpressing mice identified GPR17 as a potent negative regulator of oligodendrocyte differentiation and myelination both in vitro and in vivo. Small molecule

antagonists that can selectively block GPR17 will be invaluable tools to study unknown GPR17 biology and could expedite development of therapies for diseases potentially mediated by GPR17 such as multiple sclerosis. To date, very few small molecule antagonists have been reported in the literature and pharmacological studies with the existing non-selective and less potent antagonists have been challenging. A selective GPR17 antagonist remains an unmet need for investigations into both the basic pharmacology and therapeutic potential of GPR17. To identify novel small molecule antagonists of the GPR17 receptor, we developed and validated a GPR17 calcium mobilization assay using stable hGPR17-HEK293 cells. In addition, to determine selectivity of novel compounds over the CysLT1 receptor, we developed and validated a CysLT1 calcium mobilization assay using stable hCysLT1-CHO cells. Systematic structural modification of a non-selective (GPR17 IC_{50} = 544 nM; CysLT1 IC_{50} = 2 nM) GPR17 antagonist, Pranlukast, was performed and ~50 analogs were synthesized and evaluated in GPR17 and CysLT1 calcium mobilization assays. This resulted in the identification of three novel and selective small molecule GPR17 antagonist leads - **SN-70, SN-122** and **SN-129** exhibiting ~ 1 µM GPR17 and > 10 µM CysLT1 antagonist potency. Compounds **SN-70, SN-122 and SN-129** exhibit >5000-fold reduced potency at CysLT1 and are > 10-fold selective for GPR17 over CysLT1 compared to Pranlukast. These compounds serve as excellent leads to identify more potent and selective GPR17 antagonists. Additionally, as a proof of concept, we found that treatment of isolated OPCs (from neonatal rats) with our initial lead, **SN-23** (GPR17 IC_{50} = 1229 nM, CysLT1, IC_{50} = 1088 nM) resulted in induction of MBP expression and an expansion of cellular processes of Olig2+ OPCs. This suggested the ability of GPR17 compounds to promote oligodendrocyte differentiation and myelination. Thus, developing selective GPR17 antagonists as a potential remyelination therapeutic for MS is a promising approach.

Disclosures: S. Narayanan: None. **A.M. Decker:** None. **E.C. Tonetti:** None. **V. Vasukuttan:** None. **T.L. Langston:** None. **S.P. Runyon:** None. **X. Liu:** None. **R.Q. Lu:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Preservation of retinal ganglion cells by a novel TrkB agonist antibody in a high-pressure glaucoma model

Authors: *S. REINEHR[1], N. SCHONHOVEN[1], R. M. LINDSAY[2], P. S. DISTEFANO[2], R. FUCHSHOFER[3], H. DICK[1], S. C. JOACHIM[1];
[1]Exptl. Eye Res. Inst., Ruhr-University Bochum, Bochum, Germany; [2]Zebra Biologics Inc., Concord, MA; [3]Inst. of Human Anat. and Embryology, Univ. Regensburg, Regensburg, Germany

Abstract: Purpose: Glaucomatous neurodegeneration, a common cause of blindness, is defined as loss of retinal ganglion cells (RGCs) and their axons. Current treatments aim to lower the intraocular pressure (IOP), the main risk factor of this disease, but are not ultimately neuroprotective and do not stop disease progression in all patients. Hence, we aimed to investigate the efficacy of a BDNF mimetic, a TrkB-activating antibody (ZEB85), on high-pressure glaucomatous damage that arises in CTGF transgenic mice. Thus, by a single intravitreal injection, we treated CTGF and wildtype (WT) control mice with the TrkB agonist antibody ZEB85 and investigated protective effects on the retina. **Methods:** CTGF and WT female and male mice were injected intravitreally in one eye with either ZEB85 or a control antibody (Co-ab) at 10 weeks of age. Non-injected eyes served as controls (WT-Co, CTGF-Co). At 10, 13, and 15 weeks of age, IOP was measured. Retinal cross-sections (n=6/group) were prepared at 15 weeks and immunohistology was performed with markers for retinal ganglion cells (RBPMS), astrocytes (GFAP), and TrkB. **Results:** IOP was not altered within WT-Co groups at 10 and 13 weeks. At 15 weeks, a significantly increased IOP was visible in all three CTGF groups (all $p<0.001$). RGC cell count was significantly reduced by 40% in the CTGF-Co and CTGF-Co-ab animals compared to the WT-Co and WT-Co-ab groups (all $p<0.05$). In contrast, retinae of CTGF-ZEB85 treated mice had similar numbers of RGCs as WT-Co animals and hence, significantly more RGCs than the CTGF-Co and CTGF-Co-ab groups (both $p<0.001$). As indicated by GFAP staining, there was significantly elevated macrogliosis in CTGF-Co and CTGF-Co-ab mice compared to the WT group (both $p<0.01$). In contrast, there were fewer reactive astrocytes in the CTGF-ZEB85-treated mice compared to the CTGF-Co-ab group ($p=0.004$). BDNF receptor $TrkB^+$ cells in the ganglion cell layer was significantly lower in CTGF-Co and CTGF-Co-ab retinae compared to WT-Co mice (both $p<0.05$). Conversely, the number of $TrkB^+$ cells in CTGF-ZEB85-treated mice was significantly higher compared to CTGF-Co and CTGF-Co-ab animals (both $p<0.01$). **Discussion:** We show that intravitreal treatment of CTGF transgenic mice with ZEB85 protects from RGC loss and reduces reactive gliosis without influencing IOP. Further, more TrkB was observed in treated animals. The results suggest that activation of TrkB via an agonist antibody that mimics the native ligand BDNF could prevent glaucoma related RGC damage and may therefore serve as new therapeutic approach for glaucoma.

Disclosures: S. Reinehr: None. **N. Schonhoven:** None. **R.M. Lindsay:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Zebra Biologics Inc. **P.S. DiStefano:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Zebra Biologics Inc.. **R. Fuchshofer:** None. **H. Dick:** None. **S.C. Joachim:** None.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Compound 4f, a novel brain-penetrant reversible monoacylglycerol inhibitor, ameliorates neuroinflammation, neuronal cell loss and cognitive impairment in mice with kainic acid-induced neurodegeneration

Authors: N. ARIMURA, C. MAEDA, K. AOYAMA, N. YAMAGUCHI, A. SUGIURA, Y. TAKAHASHI, R. MAEDA, M. KAMATA, *H. MATSUI, M. TANAKA;
Takeda Pharmaceut. Co., Takeda Pharmaceut. Co., Fujisawa-Shi, Japan

Abstract: Neuroinflammation, which is a hallmark of neurodegenerative diseases, is associated with neuronal cell loss and cognitive dysfunction. Monoacylglycerol lipase (MAGL) is involved in neuroinflammation in the brain via the degradation of endocannabinoid 2-arachidonoylglycerol into arachidonic acid, a precursor of some eicosanoid; therefore, MAGL inhibitors are expected to have anti-inflammatory effects and to be novel therapeutics for neuroinflammation. We recently developed a reversible, selective, CNS penetrant, and orally available MAGL inhibitor compound **4f**. Compound **4f** at 1 mg/kg robustly increased 2-arachidonoylglycerol levels and decreased arachidonic acid levels in the mouse brain. The time course of changes in the brain 2-AG levels reflected the brain pharmacokinetics of compound **4f**. To understand the occupancy of MAGL by compound **4f**, we conducted a competition assay using T-211 which is the distinct chemical series from compound **4f**. The occupancy ratio of compound **4f** increased in a dose-dependent manner and reached a maximum occupancy of over 99% at 1 mg/kg. The result of occupancy study reflected the dose-dependent increase in brain 2-AG and the decrease in brain AA levels. To examine whether compound **4f** suppresses neuroinflammation and neuronal cell loss, we used kainic acid (KA)-injected mice that show neuroinflammation and neurodegeneration. Compound **4f** significantly decreased expression levels of TNFalpha, IL-1beta, IL-6 and MCP-1 which were elevated by the treatment with KA. Immunohistochemistry using NeuN antibody and TUNEL assay demonstrated that compound **4f** significantly suppressed neuronal cell loss in the hippocampus of the KA-injected mice. Compound **4f** also ameliorated cognitive impairment in the mice. The effects of compound **4f** on neuroinflammation and neurodegeneration were supported by gene expression profiles and pathway analysis revealing that compound **4f** reversed the KA-induced changes in the expression level of genes related to neuroinflammation and neurotransmission. The cannabinoid receptor 1 antagonist AM251 and cannabinoid receptor 2 antagonist AM630 partly blocked the neuroprotective effects of compound **4f** in the hippocampus of KA-injected mice, suggesting that CB1R and CB2R signaling contributes to neuroprotective effects of compound **4f**. Taken together, these results indicate that MAGL inhibitor compound **4f** has potential therapeutic effects in neurodegenerative diseases accompanying neuroinflammation.

Disclosures: **N. Arimura:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **C. Maeda:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **K. Aoyama:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **N. Yamaguchi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **A. Sugiura:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **Y. Takahashi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **R. Maeda:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Kamata:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **H.**

Matsui: A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited.
M. Tanaka: A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited.

Poster

622. Neuroprotection III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 622.23

Topic: C.08. Ischemia

Support: NIH-NINDS R01 NS113921

Title: Toxic Conformer and Intrinsically Disordered Prion-Like Proteins Rapidly Accumulate in Neonatal Human and Piglet Encephalopathies and iPS Cell-Derived Neuroprogenitor Models of Excitotoxicity

Authors: *L. J. MARTIN[1], M. NIEDZWIECKI[1], S. BROWN[1], V. OLBERDING[1], B. LESTER[1], N. RIVERA-DIAZ[1], A. AMREIN ALMIRA[1], C. JAVDAN[1], M. CHEN[1], C. O'BRIEN[1], S. ADAMS[1], E. KULIKOWICZ[1], F. NORTHINGTON[1], P. KRATIMENOS[2], J. LEE[1];
[1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Children's Natl. Med. Ctr., Wahington, DC

Abstract: Neonatal brain injury evolves within neural systems. The mechanisms are unknown. Neonatal piglets stereotaxically received NMDA receptor-mediated excitotoxic brain injury from quinolinic acid (QA) injection or global hypoxia-ischemia (HI) with survivals of 20-96 hours. Other piglets received HI+QA injections into neocortex and thalamus and survived for 48 hours. Additional piglets received treatments of HI+normothermia, HI+overnight hypothermia, sham+normothermia, or sham+overnight hypothermia with survival for 29 hours or 2-7 days. Control piglets received stereotaxic injection of vehicle, sham procedure, or were naïve with no anesthesia. Brains were examined using immunohistochemistry and immunoblotting for pathological conformers of alpha-synuclein (Syn), nitrated-Syn and aggregated-Syn, and misfolded superoxide dismutase-1 (mSOD1). Postmortem human neonatal hypoxic-ischemic encephalopathy (HIE) and non-HIE brains were also analyzed. Immuno-positive cells and processes were counted in neocortex, white matter, thalamus, and cerebellum. Human induced pluripotent stem (iPS) cell-derived oligodendrocyte and neuronal progenitors underwent directed-differentiation, QA treatment, and cell lysis for immunoblotting. In all neonatal piglet brain injury models, nitrated-Syn, aggregated-Syn, and mSOD1 were detected in forebrain and hindbrain postinjury as early as 20 hours by immunohistochemistry and 48 hours by immunoblotting. Compared to scant detection in control piglets, HI and QA piglets had nitrated-Syn in cortical neurons (35-45 cells/mm^2) and terminals (14,285-28,571 boutons/mm^2); aggregated-Syn was concentrated in terminals (37,142-71,428 boutons/mm^2). mSOD1 appeared in dendrites (155 processes/mm^2), neuronal cell bodies (23-50% of total neurons in cortex, thalamus, and cerebellum), oligodendrocytes (33% of total), and neocortical plaque-like

structures (11.1 lesions/mm^2). All conformers were detected in neurons and glia in human HIE brains. Human oligodendrocytes and neurons in cell culture accumulated toxic conformer proteins when injured excitotoxically. This developmental proteinopathy occurred concurrently with proteasome abnormalities. We conclude that HI and excitotoxicity acutely induce proteasome dysfunction and the formation of toxic conformer and intrinsically disordered prion-like proteins in neonatal brain. This seeding and accumulation of proteinopathy in gray matter and white matter could be trans-cellular. Synapses and oligodendrocytes that accumulate these aberrant toxic proteins could drive connectome spreading and neural system damage in neonatal brain.

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Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.01

Topic: B.09. Glial Mechanisms

Support: HKU Seed Funding for Basic Research: 202011159177
HKU Seed Funding for Basic Research: 202111159215

Title: Blood vasculature facilitates macrophage migration from the periphery into the A152T tau zebrafish brain after peripheral LPS challenge via mechanisms involving interleukin-34 (il-34) and colony-stimulating-factor-1a (csf1a)

Authors: *S. S. H. YEUNG[1], J. Y. S. HO[2], R. C. C. CHANG[1,3];
[1]Lab. of Neurodegenerative Diseases, Sch. of Biomed. Sciences, LKS Fac. of Med., The Univ. of Hong Kong, Hong Kong, China; [2]Sch. of Nursing, The Hong Kong Polytechnic Univ., Hong Kong, China; [3]The Univ. of Hong Kong, State Key Lab. of Brain and Cognitive Sci., Hong Kong, China

Abstract: The neuroinflammatory response is a vital protective mechanism within the pathogenic brain. Neurodegenerative disorders (i.e. FTD/ALS) characterized by abnormal tau deposition (termed tauopathies) are primarily associated with increased microglia response and subsequent cytokine release. Nevertheless, active involvement of peripheral immune cells such as macrophages have also been noted to promote neuroinflammation. Despite these early observations, mechanistic understanding of how macrophages are recruited to the brain and can influence tau pathological burden remain largely unclear. By using intraperitoneal injections of LPS into the zebrafish, we demonstrate increased population and migration rate of macrophages from the periphery to the brain parenchyma of both larvae and adults. Of interest, macrophage

migration is directed specifically to the head region and associated with increased proliferative microglia as shown by colocalizations between BrdU and LCP-1. Live confocal imaging of fluorescent double transgenic mpeg1^{+}/kdrl^{+} zebrafish demonstrate intimate colocalization between macrophage and blood vasculature of the zebrafish brain. Further 3D reconstruction demonstrates increased blood vessel-associated macrophages and microglia within the brain parenchyma upon peripheral LPS challenge, particularly in A152T tau-associated transgenic zebrafish. Intriguingly, RT-PCR of brain and blood fractions in A152T tau-associated zebrafish demonstrated specialized increase in il34 and csf1a, respectively. These results suggest that il34 is likely involved in macrophage/microglia proliferation, while csf1a promotes macrophage migration from periphery to the brain. Our preliminary data suggests an important role of macrophages in promoting peripheral and neuroimmune crosstalk via blood vasculature and modulated by il-34 and csf1a. Further experiments will seek to generate il-34 and csf1a knock-outs using CRISPR-Cas9 to better study their putative roles in modulating macrophage proliferation and migration in the pathogenic brain.

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Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.02

Topic: B.09. Glial Mechanisms

Support: NIH Grant NS110768

Title: Kv1.3 inhibition mitigates inflammation-sensitized hypoxia-ischemia brain injury in newborn

Authors: *J. DI LUCENTE[1], Y.-J. CHEN[2], H. WULFF[2], I. MAEZAWA[1], L.-W. JIN[1]; [1]Univ. of California, Davis, Sacramento, CA; [2]Univ. of California, Davis, Davis, CA

Abstract: Intrapartum-related neonatal encephalopathy (NE) is estimated to affect3 per 1,000 live births globally, causing 287,000 deaths and leaving a large number of survivors with serious long-term neurological deficits including cerebral palsy. There is a dearth of effective therapy. To study this form of brain injury we employedthe mouse model of neonatal lipopolysaccharides-sensitized hypoxic-ischemic brain injury (LPS-HI), which replicates a major form of NE in which perinatal infection/inflammation sensitizes the brain to subsequent HI insult and augments brain injury. Previous studies suggest that brain damage in LPS-HI requires activation of mononuclear phagocytes (MPs, including microglia and macrophages). Because we previously found that the voltage-gated potassium channel Kv1.3, preferentially expressed in MPs, is required for the pro-inflammatory and neurotoxic activation of MPs in models of adult stroke and intracerebral LPS-induced neuroinflammation, we tested whether Kv1.3 inhibition would mitigate LPS-HI brain injury. We found that Kv1.3 expression was upregulated in MPs in

the hemisphere with brain injury following LPS-HI (LPS-HI MPs). Kv1.3 inhibition by genetic knockout or a specific inhibitor called PAP-1 mitigated neuronal loss, reduced MP accumulation and the levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. Kv1.3 inhibition also improved motor coordination measured by rotarod. Surprisingly, we could not detect cell surface Kv1.3 activity in LPS-HI MPs by whole-cell patch clamp. We therefore hypothesized that LPS-HI may activate Kv1.3 in mitochondria. Indeed, flow cytometry on mitochondria isolated from LPS-HI MPs showed a ~2-fold increase in the number of Kv1.3+ mitochondria compared to sham control MPs. Confocal microscopy showed increased co-staining of mitochondrial marker MTCO1 with Kv1.3 in LPS-HI MPs. Furthermore, a mitochondria-targeting Kv1.3 inhibitor called PAPTP mitigated LPS-HI brain injury comparable to Kv1.3 knockout and PAP-1. Mitochondrial Kv1.3 expression in LPS-HI MPs was associated with decreased mitochondrial membrane potential, increased mitochondrial ROS, and decreased complex I and complex IV activities. Such mitochondrial abnormalities were rectified by Kv1.3 knockout and PAPTP. Our results suggest that mitochondrial Kv1.3 influences MP activation by modulating mitochondrial functions and is a candidate therapeutic target for NE

Disclosures: **J. Di Lucente:** None. **Y. Chen:** None. **H. Wulff:** None. **I. Maezawa:** None. **L. Jin:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.03

Topic: B.09. Glial Mechanisms

Support: endMS Personnel Award from the Multiple Sclerosis Society of Canada
CIHR Project Grant for Jason Plemel
Canada Research Chair for Jason Plemel

Title: Microglia regulate OPC recruitment and differentiation during remyelination

Authors: *C. BAAKLINI, T. LANGE, K. HIMMELSBACH, B. PHILLIPS, M. HO, K. LEE, J. PLEMEL;
Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Multiple sclerosis is an inflammatory disease characterized by the loss of myelin. Several therapies are available for people with MS that reduce disability. Yet, no treatment is available to regenerate lost myelin, a process known as remyelination. Remyelination is associated with lower disability in people with MS, highlighting the potential for remyelination-promoting therapies. Remyelination requires microglia and monocyte-derived macrophages (MDMs), but the distinction between how these cells regulate remyelination is still unknown. We hypothesize that microglia-specific ablation in an experimental model of MS will result in impaired remyelination. We induced focal demyelination by injecting the toxin LPC into the

spinal cord of mice. Microglia were ablated by injecting diphtheria toxin into mice that expressed a cre-recombinase inducible diphtheria toxin receptor due to two different inducible cre-recombinase mouse lines: CX3CR1^{CreER} and Tmem119^{CreER}. We collected tissue at 4, 7, 14, and 21 days post-lesion induction (DPL) and quantified OPC proliferation (PDGFRa, Olig2, Ki67), differentiation (CC1, Olig2) and death (acridine orange). We found that at 4DPL, OPC proliferation was impaired in the absence of microglia. By 14DPL, the absence of microglia was associated with reduced oligodendrocyte accumulation, suggesting that microglia promote OPC differentiation or survival. We also found that microglia ablation impaired remyelination using electron microscopy. Taken together, microglia regulate distinct stages of remyelination. By understanding how microglia regulate remyelination, we hope to find new ways to boost the microglial response and improve remyelination.

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Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.04

Topic: B.09. Glial Mechanisms

Support: RO1 NS106597-01A1
RO1 EY026629

Title: Dynamic temporal bio-imaging techniques reveals the interplay between vascular events and microglial responses in the progression of experimental Cerebral Malaria.

Authors: *O. D. SOLOMON[1], P. P. VILLARREAL[2], N. D. DOMINGO[3], L. F. OCHOA[4], R. STEPHENS[6], G. VARGAS[5];
[1]Dept. of Human Pathophysiology/ Translational Med., [2]Dept. of Biomed. Engin. and Imaging Sci. Group, [3]Dept. of Intrnl. Medicine, Div. of Infectious Dis., [4]Dept. of Neurosci., [5]Dept. of Neuroscience, Cell Biology, & Anat. and Dept. of Biomed. Engin. and Im, The Univ. of Texas Med. Br., Galveston, TX; [6]Ctr. for Immunity and Inflammation, Rutgers New Jersey Med. Sch., Galveston, TX

Abstract: Cerebral malaria (CM) is a lethal neurological syndrome due to a *Plasmodium spp.* infection driven by systemic inflammation. Children residing in Africa South of the Sahara are most vulnerable to developing this condition; survivors often experience neurological and behavioral sequelae. Hyperinflammation in mice deficient in the regulatory cytokine, IL-10, infected with *P. chabaudi* models important aspects of cerebral involvement. Utilizing temporal intravital fluorescence microscopy accompanied with a thin skull cranial window in IL-10$^{-/-}$ CX3CR1$^{GFP/-}$CCR2$^{RFP/-}$ mice, we reveal the interplay between the vasculature and microglia. We identified a progression of events in cerebral malaria revealing the relationships between

coagulation, microhemorrhages, and microgliosis, which have not been previously explored. Using reporters of microglia and inflammatory monocytes (IL-10$^{-/-}$ CX3CR1$^{GFP/-}$CCR2$^{RFP/-}$) and fluorescent fibrinogen we have observed microhemorrhages occurring proximal to thrombi, with the directionality of blood flow suggesting a causal link. Microhemorrhages lead to the extravasation of blood plasma proteins, such as fibrinogen promoting neuroinflammation. Our current studies have revealed that as the disease progresses, coagulation increases. Few emboli observed on day 3 post-infection are transient and do not inhibit blood flow, and microhemorrhages were not observed at this early time point while fully occluding thrombi are apparent at day 7 p.i. Prior to vessel occlusion (thrombi) and microhemorrhage, microglia are attracted to the vessels. There is a gradual increase in vessel associated microglia (VAMS) on day 3 p.i. compared to uninfected (VAMS/total microglia: 3 dpi: 50% , uninfected: 30%). While day 7 post-infection revealed a significant increase in VAMS (7 dpi: 75%,uninfected: 30%, $p<0.05$). Finally, to determine mechanisms resulting in microglial attraction to vessels, we examined CCL5 (RANTES) expression. Immunofluorescence revealed an increase in CCL5 expression on day 7 post-infection versus uninfected (Mean volume, 7 dpi: 0.10%, uninfected: 0.02%, $p<0.05$). CCL5 expression appears localized near fibrinogen with microglia associated with these sites. Altogether, our data is highly suggestive that the coagulation process becomes dysfunctional as eCM progresses resulting in thrombi blocking the flow of blood leading to microhemorrhages. Also, microglia become more associated with the vessels over time and CCL5 may play a role in attracting microglia to the vasculature.

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Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.05

Topic: B.09. Glial Mechanisms

Support: Supported by Max Planck Institute funding

Title: Absence of Fkbp51 in murine microglia attenuates response to neuroinflammatory stimulus

Authors: *S. MITRA[1], L. DILLMANN[1], L. VAN DOESELAAR[1], J. BORDES[1], M. SPRINGER[1], T. BAJAJ[2], S. NARAYAN[1], H. YANG[1], L. BRIX[1], V. KOVAROVA[1], N. C. GASSEN[2], M. V. SCHMIDT[1];
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Abstract: Microglia are one of the major types of glial cells that are essential for normal functioning of the brain. They have a range of functions that extend from protecting the neurons

from external threats like infections, to pruning synapses for proper neuronal communications. Microglia can have both anti-inflammatory as well pro-inflammatory responses - which can lead to neurodegeneration, depending upon the stimulus. Here-in we studied the role of FKBP51 - a protein that has been associated with stress response and is a modulator of glucocorticoid receptor function, in microglia. Mice with microglia specific deletion of FKBP51 were analyzed for behavioral changes using paradigms like Open Field Test, Elevated Plus Maze Test, Social Interaction Test and Home Cage Locomotion Test. They were subsequently exposed to bacterial lipo-polysaccharide (LPS) known to elicit inflammatory response and investigated for behavioral, histo-pathological and molecular changes. Analysis revealed that the microglia specific deletion led to sex dependent phenotype with male mice exhibiting reduced anxiety-like behavior along with attenuated response to LPS featured by morphological differences in Fkbp5-/- microglia as compared to controls in-vivo, which was concurrent with an in-vitro model of cultured microglia. These results not only throw light on the microglia function and homeostasis, but add to our understanding of the role of microglia in stress response on the whole.

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Poster

623. Microglia: Regulation and Function

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Program #/Poster #: 623.06

Topic: B.09. Glial Mechanisms

Support: 5R21AG058173

Title: The R47H variant of TREM2 leads to a delayed, but prolonged, inflammatory response by microglia following microstroke

Authors: *R. F. HAWKINS[1], L. J. TRIGIANI[1], A. MISTRY[2], C. B. SCHAFFER[1], N. NISHIMURA[1];
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Abstract: Cerebrovascular injury is closely associated with cognitive decline as stroke patients are at higher risk of developing dementia, while Alzheimer's disease (AD) patients present greater history of microvascular lesions. Meanwhile, the R47H variant of the triggering receptor expressed on myeloid cells 2 (TREM2) gene, expressed primarily by microglia and thought to affect the inflammatory cell response to injury, has been identified as the second greatest genetic risk factor of late-onset AD. In this study, we investigated the effect of the R47H variant on the inflammatory response of microglia to cortical microvascular injury. Using mice with the humanized R47H variant of TREM2 knocked in, we induced a microstroke in cortical arterioles with targeted photothrombosis. We monitored CX3CR1$^{GFP/+}$ microglia dynamics in R47H and

WT mice before and 1, 4, and 7 days after microstroke using two-photon microscopy (5-6 months of age, n = 14-15/group). We observed that microglia in the R47H mice were less abundant at the center of the lesion site 1 day after microstroke with microglial area coverage within 55-90µm of the lesion center being 89% lower in the R47H mice compared to WT mice (p<0.05, t-test). However, by 7 days after microstroke microglial area coverage within 200µm of the lesion site was 2.6-fold greater in R47H mice compared to WT (p<0.05, t-test), and microglia number was 21% greater than WT (p<0.05, t-test). When comparing long-term recovery after microstroke, microglia number remained 30% higher in R47H mice at 14 days after microstroke (p<0.05, t-test), but returned to levels comparable to WT by 28 days (n = 8-9/group). Using immunohistochemistry to examine differences in the expression of inflammatory and anti-inflammatory markers, and the presence of apoptotic cells in the vicinity of the lesion site (n = 3-4/group), we found that at 4 days after microstroke R47H mice had an average of 69% more expression of TNF-α (p<0.01, t-test), while at 7 days after microstroke, R47H mice had a trend towards lower expression of TGF-β1 and an average of 1.8-fold more TUNEL+ cells (p<0.05, t-test). In conclusion, we found that the R47H variant causes a delayed, but prolonged, inflammatory response to injury, resulting in increased cellular death. Overall, these results indicate that the R47H variant of the TREM2 gene leads to subtle differences in the spatial response of microglia, with differences in inflammatory cytokine expression being more pronounced. This study motivates investigation into the potential of anti-inflammatory therapeutics, such as TNF-α inhibitors, to normalize the altered inflammatory response due to the R47H variant.

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Poster

623. Microglia: Regulation and Function

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Program #/Poster #: 623.07

Topic: B.09. Glial Mechanisms

Support: NINDS F31NS127530
NIH RF1AG071587
NIH R01NS114130
NIH R01AG075820

Title: State-specific proteomic characteristics of microglia-derived exosomes

Authors: *J. V. SANTIAGO, P. KUMAR, S. RAYAPROLU, N. T. SEYFRIED, S. RANGARAJU;
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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder defined by progressive pathological protein aggregation and deterioration of cognitive function. Microglia-mediated neuroinflammation is a key pathological component of AD; however, there are critical gaps in our understanding of how microglia perpetuate AD pathology. One proposed mechanism of microglia-mediated neuroinflammation and neurodegeneration is exosome release and uptake by neighboring cells. Thus, it is possible that in AD, microglia-derived exosomes can transfer pathogenic cargo which could perpetuate pathology. However, the proteomic profiles and influence of different microglia-derived exosomal populations on AD pathology remain unknown. We hypothesize that different microglia states determine the molecular composition of exosomes. To test this, we first treated a mouse microglia cell line, BV2 cells, with various cytokines to polarize them and collect their exosomes for downstream mass spectrometry (MS) analyses. Three groups of BV2 cells (n=4/group) were treated with either lipopolysaccharide (LPS) to polarize to a pro-inflammatory state, interleukin 10 (IL-10) to polarize to a protective state, or transforming growth factor beta (TGF-β) to polarize to a homeostatic state. Untreated BV2 cells served as a control group. Following 72 hours of treatment, BV2 cells were lysed and exosomes were isolated from cell culture media. In MS studies, we identified 533 proteins in exosome fractions and 1,866 proteins in BV2 cell proteomes. We found that known exosome related proteins, Sdcbp and Igsf8, were significantly increased in the exosomal proteome compared to the whole cell proteome. Furthermore, we identified proteins that are differentially expressed across polarization states. Transmission electron microscopy images and western blotting for exosomal marker, CD9, confirmed exosome purification by our isolation method. Next, we sought to replicate these findings in primary cultures of postnatal mouse microglia. One group (n=4/group) was treated with LPS for 72 hours and the other group was left untreated. Primary microglia and isolated exosomes were lysed for MS experiments that are currently underway. Our results indicate that microglia-derived exosomes can adopt distinct state-associated protein profiles which may have differential effects on other cell types. This work will guide future studies concerning the role of exosomal cargo in perpetuating AD pathology.

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Poster

623. Microglia: Regulation and Function

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Program #/Poster #: 623.08

Topic: B.09. Glial Mechanisms

Support: NIH Grant R56AG073965

Title: Investigating the role of RUNX1 in microglial activation in the Down syndrome Dp(16) mouse model

Authors: *N. A. WILLIAMS[1], C. R. PALMER[3], C. S. LIU[5], L. RANSOM[4], J. CHUN[2];
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Abstract: Down syndrome (DS), caused by the triplication of human chromosome 21 (HSA21), is associated with cognitive deficits, including impaired short- and long-term memory. Recent studies have shown that microglia in DS brains deviate from a homeostatic state and overexpress complement cascade genes involved in synaptic pruning. Furthermore, in the Dp(16) mouse model of DS, over-active microglia cause excessive pruning of dendritic spines and cognitive deficits. The transcription factor RUNX1, located on HSA21 and expressed in microglia, is significantly upregulated in DS microglia across all ages. We hypothesized that RUNX1 overexpression in DS contributes to microglial activation that leads to excessive dendritic spine loss and cognitive abnormalities. *Runx1* expression was compared in Dp(16) transgenic mice and their wildtype littermates across the lifespan, identifying a timepoint in development during which *Runx1* commences overexpression. We then profiled primary microglia cultured from Dp(16) and wildtype mice using transcriptomic and functional assays, which established clear changes in Dp(16) microglia. This work supports the role of RUNX1 overexpression in microglia in cognitive impairments associated with DS.

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Poster

623. Microglia: Regulation and Function

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Program #/Poster #: 623.09

Topic: B.09. Glial Mechanisms

Support: NIA Grant R01AG061060-01

Title: The latent HIV reservoir from a microglia perspective

Authors: *J. SCHLACHETZKI[1], S. O'BRIEN[2], Z. OUYANG[2], S. GIANELLA[2], A. LANA[2], C. GLASS[2];
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Abstract: While antiretroviral therapy (ART) increases life expectancy for many people living with HIV, finding a cure to completely eradicate HIV has been difficult due to the virus' ability to evade ART in distant anatomical sites like the brain. There, microglia are believed to contribute to a latent reservoir of HIV that may cause low levels of chronic inflammation and viral replication, causing alterations to the synapto-dendritic compartment. Microglia may also contribute to the pathogenesis of HIV-associated neurocognitive disorder (HAND), a clinically

variable disease which develops in a significant proportion of people with HIV and can impair memory, learning, and motor skills. Little is known, however, about the transcriptomic and epigenetic profile of microglia during HIV infection and in HAND because microglia are not normally accessible from living individuals with HIV. To overcome this, we utilized rapidly autopsied postmortem brain tissue from four donors in the 'Last Gift' cohort. From the prefrontal cortex, we performed fluorescence-activated cell sorting to isolate microglia for gene expression profiling using single cell RNA sequencing (scRNA-seq), for assessment of chromatin accessibility using single cell assay for transposase-accessible chromatin with sequencing (scATAC-seq), and to evaluate active gene regulatory regions using H3K27ac chromatin immunoprecipitation sequencing (ChIP-seq). From 22,431 CD45+ cells profiled with scRNA-seq, we identified seven microglial states characterized by distinct marker genes. HIV RNA was detected in 99 of these cells, mostly in clusters associated with reactive and stressed microglia. We performed differential gene expression analysis between cells with and without detectable HIV RNA levels and found 52 upregulated genes in HIV+ cells. These genes include chemokines like CCL8, CXCL8, CCL4, and CCL3, as well as genes belonging to the AP-1 and KLF transcription factor families, indicating reactive microglia infected with HIV. We then performed scATAC-seq, and accessible HIV DNA was found in 80 out of 26,829 profiled cells, with no significant enrichment of any of the clusters. H3K27ac ChIP-seq analysis of brain tissue from people with HIV and controls showed only subtle differences in active gene regulatory regions. Taken together, we have constructed a comprehensive gene expression atlas and chromatin accessibility landscape of microglia from brains of people with HIV. Additionally, we have detected HIV RNA and DNA in microglia, and shown that microglia in HIV+ brains exist in a diverse set of activation states, despite having similar chromatin accessibility landscapes.

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Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

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Program #/Poster #: 623.10

Topic: B.09. Glial Mechanisms

Title: Inflammasome assembly as a potential mechanism of microglial activation and neuroinflammation in schizophrenia

Authors: *R. GOBER[1], R. VONTELL[2], W. SCOTT[2], L. DUQUE[2], S. GARAMSZEGI[2], D. A. DAVIS[3], A. BARREDA[2], X. SUN[2], J. DALLMEIER[2];
[1]Univ. of Miami, MIAMI, FL; [3]Dept. of Neurol., [2]Univ. of Miami, Miami, FL

Abstract: Schizophrenia (SCZ) is a debilitating and complex psychiatric disorder with a largely uncharacterized underlying biology. Several studies have proposed that immune-mediated neuroinflammation may influence SCZ onset and early progression. This inflammatory response

may be triggered by microglia, which can create many inflammatory mediators. The inflammasome complex is of particular interest in SCZ, since its assembly often occurs in microglia and has been implicated in other diseases with microglial-driven inflammation. In this study we investigate if inflammasome complex assembly may be increased in SCZ and if assembly is occurring specifically in microglia. To investigate if the inflammasome proteins are increased in SCZ, we compared postmortem brain samples taken from the superior frontal (SF), superior temporal (ST), and anterior cingulate (AC) cortices of postmortem brains with SCZ (n=16) to neurotypical controls (n=13) to identify changes in expression of the inflammasome adaptor protein, ASC (apoptosis-associated speck-like protein containing a CARD). We used immunohistochemical staining of ASC to investigate changes in the overall population of cells that express the ASC protein followed by dual labeling fluorescence of ASC and Iba-1 to determine if the ASC positive cells are microglia. Finally, to explore if full inflammasome assembly may be occurring in ASC expressing cells, we performed double-labeling of ASC and the inflammasome sensor protein, NLRP3 (NLR family pyrin domain containing 3), to look for signal colocalization. Immunohistochemical labeling and quantification of ASC-expressing cells showed significant increases (using unpaired t-tests) in the total counts of ASC positive cells in gray matter of SCZ brains across the SF (54%, p=0.0015), ST (75%, p=0.0022), and AC (68%, p=0.0002) regions compared to counts from the controls, although there were no significant changes seen in the subcortical white matter of these brain regions. The double-labeling experiments then demonstrated that ASC expression is specifically seen in Iba-1 positive microglia and that the percentage of microglia that express the ASC protein was significantly higher (using unpaired t-tests) in the superior frontal cortex of SCZ brains with 14.3% more microglia expressing ASC compared to control cases (p=0.0055). Lastly, experiments show that NLRP3 is also expressed in the same ASC expressing cells, suggesting that full inflammasome oligomerization may be occurring. These results suggest that inflammasome complex assembly in microglia may be pathologically important in SCZ and could provide new therapeutic targets for treating the disorder.

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Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.11

Topic: B.09. Glial Mechanisms

Support: NIH Grant AI073693

Title: Hmgb1-mediated microglial activation as a mechanism for cognitive dysfunction in neuropsychiatric lupus

Authors: *M. MIZRACHI, K. CARROLL, A. ZARFESHANI, N. KELLO, N. TEHRANI, B. T. VOLPE, B. DIAMOND;
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Abstract: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the presence of anti-nuclear antibodies as well as a broad array of clinical manifestations spanning nearly every organ system. Neuropsychiatric syndromes affect over one half of SLE patients, presenting commonly as cognitive dysfunction. The pathophysiology of neuropsychiatric lupus is not fully understood; however, a critical mechanism involves passage of inflammatory cytokines and anti-brain antibodies through the blood-brain barrier (BBB). We previously developed a mouse model to study the effects of anti-dsDNA antibodies that cross-react with the NMDA receptor (NMDAR), termed DNRAbs, which are present in approximately 30% of SLE patients and whose presence in the CSF is associated with non-focal CNS manifestations of neuropsychiatric lupus. In this model, non-spontaneously autoimmune mice are immunized with DWEYS peptide, a DNA mimotope that elicits production of DNRAb antibodies, which act as positive allosteric modulators of the NMDAR. LPS is given to cause BBB breach in the hippocampus. There are two stages of brain injury. The first stage, lasting up to a week, is characterized by excitotoxic neuronal death, secondary to DNRAb-mediated NMDAR activation. The second stage, lasting months, involves an inflammatory homeostasis, consisting of microglial activation, loss of dendritic arborization in surviving hippocampal neurons, and neuronal secretion of HMGB1, a chromatin protein that can be secreted to act as a damage-associated molecular pattern (DAMP). Here we show that HMGB1 acts directly on microglia, through RAGE and TLR4 signaling. HMGB1 stimulates microglia to secrete IFNa, transcriptionally upregulating interferon regulatory factors, including IRF7. IFNa induces C3 and C1q transcription in microglia. HMGB1 also stimulates microglia to secrete TNFa and IL-1b, which may enhance the inflammatory milieu in surrounding neurons. In addition, we found that the ACE-inhibitor captopril reverses dendritic pruning in DWEYS-immunized mice — an essential component of the second stage of DNRAb-related damage — by regulating expression of the inhibitory receptor LAIR1 in microglia. Whereas captopril reverses dendritic pruning in DWEYS-immunized wildtype mice, captopril has no such effect on LAIR1 knockout mice. We are currently analyzing RNA sequencing data from microglia isolated from captopril-treated, DWEYS immunized mice, to characterize the transcriptional program responsible for this process.

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Poster

623. Microglia: Regulation and Function

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Program #/Poster #: 623.12

Topic: B.09. Glial Mechanisms

Support: NIH Grant R03AG070111-01

Title: The sex-specific effects of adolescent NAc pruning on receptor concentrations and social behavior

Authors: *J. M. KIRKLAND, A. M. KOPEC;
Dept. of Neurosci. and Exptl. Therapeut., Albany Med. Col., Albany, NY

Abstract: Social interaction is a highly conserved, rewarding experience; the nucleus accumbens (NAc) is responsible for processing appetitive stimuli, including social reward. During adolescence, peer-to-peer socialization increases, concurrent with complement-mediated microglial pruning of the NAc. ***In rats, the pruning period for the NAc is sex-specific, and in both sexes, downregulates the expression of adolescent-typical social play behavior.*** In males, this effect is driven by microglial pruning of D1-dopamine receptors, but in females, there is a different, yet unknown pruning target.In this ongoing set of experiments, we use a highly specific C3-receptor antagonist to investigate the short- and long-term changes in social behavior that result from natural pruning in the NAc. We also use proteomics to better understand the molecular effects of NAc pruning, including identifying potential female pruning target(s).These studies will expand our mechanistic understanding of sex-specific effects of synaptic pruning and pruning's long-term effects on social behavior and mental health.

Disclosures: J.M. Kirkland: None. **A.M. Kopec:** None.

Poster

623. Microglia: Regulation and Function

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Program #/Poster #: 623.13

Topic: B.09. Glial Mechanisms

Support: NIH T32NS082174

Title: Novel C1q-Receptor Interactions as Regulators of Microglial Polarization

Authors: *P. SAKTHIVEL[1], A. J. ANDERSON[2];
[1]Anat. and Neurobio., [2]Physical Med. and Rehabil., Univ. of California, Irvine, Irvine, CA

Abstract: Neuroinflammation is a hallmark of all neurodegenerative conditions and can actively inhibit neuronal repair and recovery. Microglia, the immune cells of the central nervous system (CNS), contribute to this molecularly hostile environment by transitioning into an inflammatory state in response to disease or injury. Although they have the potential to be polarized into an anti-inflammatory state that is more conducive to neuronal repair, microglia often remain stuck in a pro-inflammatory state. It remains unclear how microglia transition between these phenotypes - despite the notion that microglial phenotype heavily influences neuronal repair. One potential molecular regulator of interest is C1q, the initiator molecule of the complement

cascade that has recently become recognized for novel functions within the CNS. In this study, we utilize human induced pluripotent stem cell-derived microglia (hiPS-mg) to investigate a novel role for C1q in regulating microglial polarization. Here, we show (1) exogenous C1q drives hiPS-microglia to an inflammatory state, (2) hiPS-microglia treated with lipopolysaccharide (LPS) shift to an inflammatory state and upregulate autocrine C1q production and (3) blocking this autocrine C1q upregulation significantly attenuates LPS-induced inflammation. To interrogate the mechanisms underlying this, we are investigating five novel C1q receptors that our lab recently identified (ADCY5, BAI-1, CD44, cMET, and GPR62). Bulk RNA sequencing and proximity ligation assay (PLA) have validated receptor expression and C1q-receptor candidate interactions at the microglial membrane, confirming a novel role for C1q as a ligand with hiPS-microglia. Indeed, our data suggests these novel C1q receptors play a role in regulating microglial inflammation and polarization. Further characterization of C1q-receptor interactions and their influence on microglial state will elucidate the mechanisms underlying microglial inflammation, allowing for the identification of therapeutic targets that can alleviate inflammation in a wide range of neurodegenerative disorders.

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Poster

623. Microglia: Regulation and Function

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Topic: B.09. Glial Mechanisms

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NIH DA052209
NIH DA026306 (P5)

Title: Elucidating the role of ephrin-B1 in Interferon Beta mediated neuroprotection in HIV associated neurocognitive disorder (HAND)

Authors: *J. KOURY[1], H. SINGH[1], S. SUTLEY[1], D. FOK[1], B. GELMAN[2], I. ETHELL[1], M. KAUL[1];
[1]Univ. of California Riverside, Riverside, CA; [2]Univ. of Texas Med. Br., Galveston, TX

Abstract: 37 million people worldwide are diagnosed with Human Immunodeficiency Virus (HIV). Of these 37 million, an estimated 15-55% of people living with HIV develop HIV-associated neurocognitive disorder (HAND). Previous work in ours and other labs identifies a transient increase of interferon beta (IFNβ), an anti-inflammatory and antiviral type 1 interferon, preceding any behavioral or neuropathological signs in the HIVgp120 transgenic mouse and SIV models, suggesting a neuroprotective role for IFNβ in early HIV infection. We have previously

shown that exogenous intranasal exposure of HIVgp120tg animals with IFNβ is sufficient to confer neuroprotection. Here, we describe the ability of IFNβ to regulate an important cell surface transmembrane ligand, ephrin-B1, in the CNS. Neuronal ephrin-B1 has been well studied and implicated in neuronal development, recruitment of NMDA receptors and synaptic plasticity. However, the regulation of ephrin-B1 as well as ephrin-B1's role in HIV has not been well characterized. In this study, we identify a significant positive correlation between type 1 interferon master regulator, IRF7, and ephrin-B1 mRNA in the cortex of HIV+ Patients. In addition, we describe significant inverse correlations between ephrin-B1 mRNA in the cortex of HIV+ Patients with both neurocognitive domain scores and neuronal gene expression. In a transgenic NeuroHIV mouse in vivo model, we show in cortex and hippocampus that abrogating IFNβ signaling decreases RNA expression of ephrin-B1. Conversely, intranasal treatment with IFNβ induces ephrin-B1 protein expression in a cell type specific manner within the hippocampus and partially in the cortex in non-transgenic control animals. Taken together, this data suggests that IFNβ may be an important regulator of the ephrin system, potentially implicating the ephrin system in IFNβ mediated protection of HIV mediated neuronal damage.

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Poster

623. Microglia: Regulation and Function

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Support: AMC Start-Up Funds
NIA; R03AG07011

Title: Social behavior development is altered by adolescent morphine exposure: Importance of microglia

Authors: *D. K. NDAMBUKI, L. NTI-KYEREMEH, A. KOPEC;
Albany Med. Col., Albany Med. Col., Albany, NY

Abstract: Introduction: Social behavior development during adolescence is mediated by the nucleus accumbens via sex-specific mechanisms of synaptic pruning. Synaptic pruning is the phagocytosis of synapses or synaptic components primarily by microglia, the resident immune cells in the nervous system. Many psychiatric disorders contain a social component to their pathophysiology and previous studies have shown that adolescent opioid exposure can increase mental disorder incidence later in life. **Hypothesis:** We hypothesize that adolescent morphine exposure will increase synaptic pruning leading to adult social deficits. **Methods:** (1). Microglia were isolated and exposed to varying concentrations of morphine *ex vivo* and (2). Social behavior tests were performed on adult rats that were exposed to morphine during adolescent

NAc pruning period. **Results:** We found that (1). morphine dose-dependently increases phagocytosis by microglia *ex vivo* and (2). adolescent morphine exposure leads to adult social deficits. **Discussion:** We demonstrate that social deficits are observed after opioid exposure restricted to the NAc pruning period. Furthermore, microglia increase phagocytosis in response to morphine in culture, supporting our predicted influence of opioids on microglia. *In vivo* analysis of NAc synaptic pruning after adolescent morphine exposure is ongoing. Adolescent drug exposure is known to increase substance use disorder risk; these data suggest adolescent opioid exposure may also interfere with social behavior development via abnormal immune signaling.

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Poster

623. Microglia: Regulation and Function

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.16

Topic: B.09. Glial Mechanisms

Support: Hope College
Ed and Ann Anderson Neuroscience Award

Title: Characterization of the microglial response dynamics in the zebrafish olfactory system following injury

Authors: *N. W. VORHEES, E. CALVO-OCHOA;
Biol., Hope Col., Holland, MI

Abstract: Zebrafish (Danio rerio) presents 16 neurogenic niches and maintains the ability to regenerate neurons (i.e., neurogenesis) throughout its lifespan. This makes the zebrafish an excellent candidate to study brain repair and regenerative mechanisms, contrary to mammalian models that do not display extensive repair mechanisms or neurogenesis following damage. The olfactory system of zebrafish, composed of the olfactory bulbs (OB) and olfactory epithelium (OE) serves the sense of smell by mediating the recognition and response to odorants. These structures, coupled with the adjacent telencephalic ventricular zone (VZ), present remarkable plasticity, repair, and regeneration following damage. It has been suggested that neuroinflammatory processes underlie some of these regenerative processes. In particular, microglia - the primary immune cell of the central nervous system - play a central role in regulatory behavior of the inflammatory response. Microglia have been shown to be an important modulator in neural plasticity, repair, and regeneration following injury in some regions of the zebrafish brain. However, it is not known whether neuroinflammatory mechanisms are involved in plasticity and repair in the olfactory system. Here, we investigate microglial responses following lesions and regenerative responses in the olfactory bulb of zebrafish. In this study, we used a focal excitotoxic lesion in the zebrafish olfactory bulb to investigate recovery mechanisms

and the microglial response in the olfactory system. We used adult zebrafish of both sexes and injected 1 µl of 15 mM quinolinic acid (QA) into the right olfactory bulb producing a unilateral focal excitotoxic lesion. We assessed microglial activation, migration, and phagocytosis via immunohistochemistry. At 1 day post lesion (dpl) we found: 1) a dramatic increase in number of microglial cells in the ipsilateral OE and OB, and in the VZ; 2) a robust activation into an amoeboid phagocytic state on the ipsilateral side of lesion; 3) active migration both rostrally from the peripheral nervous system and caudally; and 4) phagocytic profiles localized near degenerating olfactory axons. We also observed that at 21 dpl: 5) microglial profiles return to control levels; 6) microglia return to inactivated states in the OE and OB; while 7) persistent microglial activation is still observed in the VZ. This study is one of the few to characterize microglial responses in the olfactory bulb of zebrafish as a consequence of olfactory bulb lesions, contributing to the growing field of knowledge of microglia's role in underlying neuroprotective and neuroregenerative processes.

Disclosures: N.W. Vorhees: None. **E. Calvo-Ochoa:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.17

Topic: B.09. Glial Mechanisms

Support: NeuroNET

Title: Do microglia contribute to brain region differences in excitotoxic susceptibility?

Authors: *D. ACHARYYA[1], R. A. PROSSER[2];
[1]The Univ. of Tennessee, Univ. of Tennessee, Knoxville, Knoxville, TN; [2]Biochem. & Cell. and Mol. Biol., UNIV. TENNESSEE, Knoxville, TN

Abstract: A variety of pathological conditions, including traumatic brain injury, stroke and neurodegenerative diseases, generate excitotoxic conditions that ultimately result in substantial cell death and damage. However, several studies, including recent data from our lab, support the suprachiasmatic nucleus (SCN) being more resistant to excitotoxic damage in response to over-activating glutamate receptors compared to other cells and/or brain regions. Given that microglia have been shown to participate in the neural responses to excitotoxic stimuli, we are investigating whether differences in baseline and/or stimulated microglia may contribute to the SCN's excitotoxic resiliency. Acute coronal brain slices containing either the SCN + surrounding anterior hypothalamus (AH) or the cortex prepared from adult male C57Bl/6 mice were either left untreated or exposed to 10 mM NMDA for 1 h beginning at Zeitgeber time (ZT) 6 (ZT 0 = lights-on; ZT 12 = lights-off). Slices were then maintained for an additional 3 h control medium. Our previous work has shown that the SCN is more resilient to excitotoxic damage under these conditions compared to both the AH and cortex. The brain slices were then fixed, sectioned, and

stained for the pan-microglial marker IBA1 to assess the morphology and number of microglia in the three brain regions. Sections were imaged using a fluorescent microscope and the images were analyzed using ImageJ. Our preliminary data show no significant differences in either the morphology or the number of microglia across the 3 brain regions under control conditions. However, NMDA induced a significant shift in microglia morphology to a more amoeboid shape in the SCN but not in the AH or the cortex. NMDA also increased the number of microglia in the cortex when compared to both the SCN and the AH and when compared to the cortex under control conditions. Thus, SCN, AH and cortex microglia appear to respond differently to a robust excitotoxic stimulus under ex vivo conditions. We are continuing to investigate differences in microglia across these three brain regions under varying conditions. Identifying mechanisms underlying SCN excitotoxic resiliency could guide future research into improved therapies for these neuropathological conditions.

Disclosures: D. Acharyya: None. **R.A. Prosser:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.18

Topic: B.09. Glial Mechanisms

Title: The Effect of Acute Cannabis Exposure on Microglial Density, Distribution and Spacing is Region Dependent in Adult, Male Mice

Authors: *H. A. VECCHIARELLI[1], H. THORPE[2], S. LOEWEN[1], C. MURRAY[1], H. KAYIR[2], J. KHOKHAR[2], M.-È. TREMBLAY[1];
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Abstract: Microglia, the brain's resident immune cells, are increasingly becoming recognized for their physiological roles as well as their immunological ones. Microglia possess cannabinoid receptors and respond to cannabinoids. Administration of the primary phytocannabinoids, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), is considered anti-inflammatory, but this is dependent on the route of administration (e.g. injection, ex vivo), duration and compounds delivered. To date, no one has investigated how another relevant route of administration, cannabis inhalation, affects microglia physiological functions.
Cannabis was administered to adult, male, C57BL/6J mice for 15 min (one 15 second puff every 5 min; 3 puffs total; 0.15 g flower/puff). Four groups were utilized, mice that received control air vapor, and mice that were exposed to either: high CBD/low THC, high THC/low CBD, or balanced THC/CBD cannabis strains. Brains were isolated 30 min post-cannabis administration onset, when THC levels peak in the brain. We stained the tissue with antibodies against IBA1 (microglia and macrophages) and TMEM119 (more specific for microglia) and looked at IBA1+ cell density, nearest neighbor distance and spacing index (changes in number and distribution), in regions important for cognition, memory, and emotional regulation.

In the basolateral amygdala, there were no changes in density or nearest neighbor distance, but there was lower spacing index in the CBD versus THC strain—this could mean there is a change in the location of IBA1+ cells resulting from migration without a change in their number. In the hippocampus dentate gyrus molecular layer, there was increased density in the balanced versus the THC strain, and also potentially in the CBD versus the THC strain—indicating that CBD may increase IBA1+ cell density. In the subgranular zone there was an increase in spacing index in the CBD versus the THC strain. We found no differences in these parameters in either the prelimbic or infralimbic cortices.
Our preliminary results indicate that the effect of acute cannabis exposure on microglia is dependent on region. Confocal microscopy work will assess the colocalization of IBA1 with TMEM119 to confirm that these cells are microglia, and assess the morphology of these cells. We will use scanning electron microscopy to investigate potential changes in microglial organelles and interactions with parenchymal elements. This work will lay the foundation for understanding how vaporized cannabis exposure alters microglial form and function, and determining how these parameters change with chronic exposure and in response to stress, infection or disease.

Disclosures: H.A. Vecchiarelli: None. **H. Thorpe:** None. **S. Loewen:** None. **C. Murray:** None. **H. Kayir:** None. **J. Khokhar:** None. **M. Tremblay:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.19

Topic: B.09. Glial Mechanisms

Title: Sat1 alleviates neuropathic pain by regulating microglia activation

Authors: *J. SHIN, S. KIM, H. SHIN, D. KIM;
Chungnam Natl. Univ. Col. of Med., Chungnam Natl. Univ. Col. of Med., Daejeon, Korea, Republic of

Abstract: Microglia are glial cells and responsible for the immune defense and inflammatory responses in the central nervous system (CNS). The activation of microglia has been considered to develop and maintenance of neuropathic pain, moreover, prolonged microglia activation can cause chronic neuroinflammation and increase production of pro-inflammatory mediators. Here, we succeeded in alleviating neuropathic pain by inhibiting the microglial activation through the downregulation of SAT1 with PLGA nanoparticles. To relive neuropathic pain behavior, SAT1 shRNA encapsulated PLGA nanoparticles (shSAT1 NPs) were prepared and characterized. We revealed that shSAT1 NPs significantly inhibited excessive production of TNF-α and IL-1β in LPS incubated BV2 cells. In addition, neuropathic pain induced by spinal nerve injury was effectively reduced in rats and mice, and the decrease of microglia activity was also confirmed by histological and cytokine analysis. Taken together, these data suggest that SAT1

downregulation in microglia with PLGA nanoparticles efficiently relives neuropathic pain, and it would be therapeutic value for treating neuropathic pain.

Disclosures: **J. Shin:** None. **S. Kim:** None. **H. Shin:** None. **D. Kim:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.20

Topic: B.09. Glial Mechanisms

Support: NRF-2020R1C1C1007488
NRF-2020R1I1A1A0105309611
NRF-2021R1C1C2007218

Title: Perampanel reduces brain damage via induction of M2 microglia in a neonatal rat stroke model

Authors: H. SHIN[1,5], ***K. LEE**[1,6,2], J. KANG[2,3], S. CHOI[1,2], D. KIM[1,4,2], Y. YI[7];
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Abstract: Purpose: Ischemic stroke is a leading cause of death and disability worldwide. Additionally, neonatal ischemia is a common cause of neonatal brain injury, resulting in cerebral palsy with subsequent learning disabilities and epilepsy. However, there is currently a lack of effective treatments available for patients with perinatal ischemic stroke. In this study, we investigated the effect of Perampanel (PER)-loaded poly lactic-co-glycolic acid (PLGA) by targeting microglia in perinatal stroke.**Methods:** After formation of focal ischemic stroke by photothrombosis in P7 rats, PER-loaded PLGA was injected intrathecally. Proinflammatory markers (TNF-α, IL-1β, IL-6, COX2, and iNOS) and M2 polarization markers (Ym1 and Arg1) were evaluated. And we investigated whether PER increased M2 microglial polarization in vitro.**Results:** PER-loaded PLGA nanoparticles decreased the pro-inflammatory cytokines compared to the control group. Furthermore, they increased M2 polarization.**Conclusion:** PER-loaded PLGA nanoparticles decreased the size of the infarct and increased motor function in a perinatal ischemic stroke rat model. Pro-inflammatory cytokines were also reduced compared to the control group. Finally, this development of a drug delivery system targeting microglia confirms the potential to develop new therapeutic agents for perinatal ischemic stroke.
Keywords: Ischemic stroke, Neonate, PLGA, Nanoparticle, Perampanel, Microglial polarization

Disclosures: **H. Shin:** None. **K. Lee:** None. **J. Kang:** None. **S. Choi:** None. **D. Kim:** None. **Y. Yi:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.21

Topic: B.09. Glial Mechanisms

Title: Amitriptyline repositioning prolongs analgesic effect by enhanced targeting microglia

Authors: *S. KIM, J. SHIN, N. SHIN, H. SHIN, D. KIM, S. LEE;
Chungnam Natl. Univ. of Med., Chungnam Natl. Univ. of Med., Daejeon, Korea, Republic of

Abstract: Amitriptyline (AMI) has been applied to various pain conditions, including neuropathic pain, but the clinical outcomes remain unsatisfactory, and the underlying molecular mechanisms are unclear. Therefore, we investigated the development of a drug repositioning for neuropathic pain based on low dose of AMI encapsulated in poly (D, L lactic-co-glycolic acid) (PLGA) nanoparticles (AMI NPs), since PLGA nanoparticles are known to be enhancing delivered to microglia. The analgesic effects of AMI and AMI NPs on neuropathic pain were evaluated with behaviors and inflammatory responses using a rat model of spinal nerve ligation (SNL). Analgesic effect on SNL-induced neuropathic pain was persisted for 4 h in AMI-injected rats. By contrast, AMI NPs significantly alleviated mechanical allodynia for 3 days. Both microglial activation and pro-inflammatory mediators were notably reduced in the spinal dorsal horn, according to histological and cytokine analyses. This study showed that AMI NPs can provide a prolonged analgesic effect by enhanced targeting of microglia. Moreover, neuropathic pain was improved by AMI NP-mediated regulation of pro-inflammatory cytokine release from activated microglia. Our view implies that sustained release of AMI from microglia-targeted NPs provides drug repositioning along with long-term analgesic effects while reducing potential side effects of the drug.

Disclosures: S. Kim: None. **J. Shin:** None. **N. Shin:** None. **H. Shin:** None. **D. Kim:** None. **S. Lee:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.22

Topic: B.09. Glial Mechanisms

Title: Microglia-specific knockout of Fmr1 leads to FXS-like behavior deficits

Authors: *H. LI[1], B. XU[2];
[1]UF Scripps Biomed. Research, Univ. of Florida, [2]UF Scripps Biomed. Research, Univ. of Florida, Jupiter, FL

Abstract: Fragile X syndrome (FXS) is the most common heritable intellectual disability and the biggest contributor to monogenic autism spectrum disorders. It's caused by mutations in the Fmr1 gene, leading to transcriptional silence and absence of the encoded protein FMRP. As an RNA binding protein, FMRP controls transportation and translation of its target mRNAs, including synapse-located mRNAs. Despite of its known functions in synaptic transmission, there is no approved drug for the treatment of FXS. Microglia are the resident immune cells in the CNS and play important roles in neurodevelopment; however, it's unknown whether and how microglia contribute to the development of FXS. In the present study we found that in wildtype mice Fmr1 expression in microglia started between E14.5 and E16.5. The protein level in microglia peaks during the first postnatal week and declines with age. FMRP is undetectable immunohistochemically in adult microglia. We generated a mouse strain to selectively delete Fmr1 in microglia in the 1st postnatal week. The Fmr1 cKO mice displayed FXS-featured behavior deficits, including audiogenic seizure, hyperactivity, and impairments in working memory and sociability. Fmr1 knockout led to enlarged microglia size in multiple brain regions, including hippocampus, striatum and inferior colliculus. In the hippocampus of male Fmr1 cKO mice at P14 microglia had fewer phagocytic cups compared with control mice. Consistently, engulfment of postsynaptic structure by microglia was decreased in male cKO mice. The size of dendritic spines on CA1 neurons was smaller in adult Fmr1 cKO mice of both sexes. To reveal the mechanism underlying the increased susceptibility of audiogenic seizure, we focused on the inferior colliculus, a brain region critical for auditory information processing. Parvalbumin neurons decreased by 30% in male cKO mice compared with their littermate controls, while in female cKO mice, we observed elevated number of inhibitory synapses. Both of changes potentially cause E/I imbalance and hypersensitivity of the auditory pathway. Taken together, our study showed that Fmr1 was expressed in microglia in young mice. Selective knockout of Fmr1 in microglia leaded to impaired microglia functions and FXS-featured behavior deficits.

Disclosures: H. Li: None. **B. Xu:** None.

Poster

623. Microglia: Regulation and Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 623.23

Topic: B.09. Glial Mechanisms

Support: NHMRC Grant GNT1163981 (Australia)
MRC Grant MR/M019969/1 (UK)
The Mater Foundation
Australian Government Research Training Program Stipend Scholarship & Tuition Fee Offset

Title: A Colony Stimulating Factor 1 Receptor (CSF1R)-related leukoencephalopathy-causing mutation alters microglial morphology and CSF1R signalling but does not impact experimental autoimmune encephalitis onset.

Authors: *J. STABLES[1], N. KHAN[2], T. WOODRUFF[2], K. IRVINE[1], D. HUME[1];
[1]Mater Res. Inst., [2]Sch. of Biomed. Sci., Univ. of Queensland, Brisbane, Australia

Abstract: Colony Stimulating Factor 1 Receptor (CSF1R) signalling is crucial for the differentiation, maintenance and proliferation of tissue resident macrophages, including microglia. In 2011 a group of patients with an early-onset dementia were identified as having a heterozygous mutation in *CSF1R*. Patients with this dementia, now referred to as CSF1R-related leukoencephalopathy, have reduced microglial density and loss of the homeostatic microglial phenotype. Disease-associated mutations typically occur in the tyrosine kinase domain of the receptor, preventing autophosphorylation and activation of downstream signalling. The literature has debated whether the disease results from haploinsufficiency or a dominant-negative effect of the mutant CSF1R.

To investigate this in a preclinical setting, we created a novel mouse model with a single copy of a human-equivalent CSF1R mutation by altering amino acid 631 in mouse CSF1R from glutamic acid to lysine (*Csf1r-E631K*). These mice (*Csf1r*$^{m/WT}$) have drastically reduced CSF1R signalling in response to CSF1 treatment compared to *Csf1r*$^{WT/WT}$ mice. However, unlike patients, they do not develop ventricular enlargement, corpus callosum thinning or motor symptoms. Histological comparison of *Csf1r*$^{m/WT}$ and *Csf1r*$^{WT/WT}$ brains at 3, 9, and 43 weeks of age revealed that mice with the mutation had significantly fewer microglia in all brain regions examined. Their microglia had increased soma size and reduced average total process length per cell, indicating a more activated phenotype.

To determine the impact of the Csf1r-E631K mutation in the setting of neurological challenge, we next sought to investigate whether the *Csf1r-E631K* mutation altered the onset and progression of the rodent model of multiple sclerosis (MS), Experimental Autoimmune Encephalitis (EAE). Microglia are thought to play roles in both active inflammation of MS lesions and in the remyelination of axons. Inhibiting CSF1R signalling to deplete microglia has been proposed as a therapeutic strategy for MS. EAE was induced in female *Csf1r*$^{m/WT}$ and *Csf1r*$^{WT/WT}$ mice, aged 8-12 weeks. Clinical scoring and rotarod testing were used to determine disease progression. Symptoms manifested on day 9 (post EAE induction) for mice of both genotypes. The cumulative clinical score and rotarod scores of mice that remained on day 23 were not significantly different. Histological investigations are ongoing.

The initiation of MS was neither inhibited nor exacerbated in a CSF1R-related leukoencephalopathy mutant mouse with impaired CSF1R signalling and microglial development.

Disclosures: **J. Stables:** None. **N. Khan:** None. **T. Woodruff:** None. **K. Irvine:** None. **D. Hume:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NMSS RG-1807-31964
NMSS RG-1508-05912
NIH NINDS NS112727
NIH NIAID AI144004
HFHS A10270
HFHS A30967
NIH NIAID AI145829-03

Title: Benzene exposure during pregnancy increases experimental autoimmune encephalomyelitis severity in offspring

Authors: *A. NGUYEN[1,2,3,4], A. HU[2], J. S. TOOR[4], A. MAXWELL[2], M. NEMATULLAH[3], J. DING[2], G. MOR[2], S. GIRI[3];
[1]Wayne State Univ. Translational Neurosci. Program, Detroit, MI; [2]Dept. of Obstetrics and Gynecology, C.S. Mott Ctr. for Human Growth and Develop., Detroit, MI; [3]Dept. of Neurol., Henry Ford Hlth. Syst., Detroit, MI; [4]Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: Although Multiple Sclerosis (MS) is one of the most common autoimmune demyelinating diseases, its exact etiology is still unknown. Research in the field of Developmental Origins of Health and Disease has shown environmental toxin exposure during pregnancy causes maternal inflammation, which can adversely impact fetal neurodevelopment and cause long-term effects on offspring health. Benzene in particular, a growing concern in urban areas, has been associated with developmental delays, psychiatric disorders, and hyperinflammation in the brain. Microglia, the primary immune mediators of the central nervous system, have been heavily implicated in pathogenesis of these conditions. We hypothesize benzene-induced in utero inflammation may predispose offspring to greater disease severity in the murine experimental autoimmune encephalomyelitis (EAE) model of MS. In this study, pregnant C57BL/6J mice were exposed to benzene inhalation chambers (50 ppm) for 5 hours/day from embryonic day 0.5-17.5. To induce EAE, female and male offspring were challenged at 6-8 weeks of age with a subcutaneous emulsion (200ug/200ul) of myelin oligodendrocyte peptide (MOG_{35-55}) and Complete Freund's Adjuvant (CFA). Control animals were given CFA only. Animals were assessed daily using an established clinical score for disease symptomology. On post-induction day 16 (PID 16), all animals were sacrificed when one group required euthanasia due to moribund paralysis. Brain samples were collected for flow cytometry and spleens were used for antigen recall against MOG. We found that average EAE clinical score was highest in benzene-exposed male offspring on PID 16. EAE groups had higher CD4+ frequency in the brain compared to CFA only groups, indicative of neurological infiltration. Flow cytometry revealed both male and female benzene EAE offspring had a greater frequency of MHCII+ microglia compared to control EAE offspring, suggesting benzene exposure leads to hypersensitivity of microglia in EAE induction. Additionally, male control EAE offspring had higher frequency of lymphocytes in the brain compared to matched females, indicating basal sex differences in response to immune challenge. There were no significant cytokine differences

between groups at 48 hours post-MOG in spleen antigen recall. Our results suggest that inflammatory in utero environments may induce dysregulation in the developing neuroimmune system in an exposure- and sex-specific manner.

Disclosures: **A. Nguyen:** None. **A. Hu:** None. **J.S. Toor:** None. **A. Maxwell:** None. **M. Nematullah:** None. **J. Ding:** None. **G. Mor:** None. **S. Giri:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Multiple Sclerosis Society
University of Iowa Carver College of Medicine
University of North Texas Health Science Center
National Institutes of Health

Title: Understanding the enigmatic neuro-reparative role of a CNS-targeting CD8 T-cell in a mouse model of Multiple Sclerosis

Authors: *K. E. BAMBINO[1], K. HERNANDEZ[1], S. B. ORTEGA[1], N. J. KARANDIKAR[2]; [1]Univ. of North Texas Hlth. Sci. Ctr., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [2]Univ. of Iowa, Iowa City, IA

Abstract: Multiple Sclerosis (MS) is a debilitating central nervous system (CNS) disease where it is believed that T-cells orchestrate the destruction of the myelin sheath surrounding a neuron's axon, leading to loss of saltatory conduction and formation of a lesion. Patients with MS present with cognitive, emotional, and mobility impairments. CD8 T-cells are the most abundant cell within and around the MS lesion irrespective of patient age, clinical subtype, disease duration, or type of immunosuppressive treatment. Contrary to current thought and work by others, our prior work has shown that CD8 T-cells - with specificity to myelin antigens - are capable of ameliorating disease *before* and *after* induction. In fact, we have observed a rapid reversal of disease that outpaces endogenous cerebral neuro-reparative mechanisms. Our hypothesis is that, in addition to the previous roles we have already elucidated, we believe myelin-reactive CD8 T-cells have a direct neuro-reparative role on cells of the CNS. Our current data shows that direct *in vitro* co-culturing of myelin antigen-reactive (**Mye-Ag**) CD8 T-cells (Mye-Ag) with progenitor CNS cells leads to an increase in mature oligodendrocytes (OLIG2+). This is a function specific to CD8 T-cells, as co-culturing CNS cells with supernatants from CD8-devoid splenocytes cultured with myelin antigen, results in loss of $OLIGO2^{+}$ cells. Our next step is to evaluate *in vivo* the induction of pro-oligodendrogenesis effector molecules in mice treated with Mye-Ag CD8 T-cells. Using the 10X Genomics Visium platform, we will focus on areas in the

adult brain known to be involved in the oligodendrogenesis process which will help explain disease reversal.

Disclosures: K.E. Bambino: None. **K. Hernandez:** None. **S.B. Ortega:** None. **N.J. Karandikar:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2018R1A6A1A03025221 from National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology

Title: Anti-neuroinflammatory response in the prefrontal cortex of rats injected with polyinosinic:polycytidylic acid (Poly-I:C) by vagus nerve stimulation

Authors: *U. NAMGUNG, K.-J. KIM;
Daejeon Univ., Daejeon, Korea, Republic of

Abstract: Injection of polyinosinic:polycytidylic acid (poly-I:C) into experimental animals induces neuroimmunological symptoms and thus has been used for the study of neurological disorders such as anxiety, depression and chronic fatigue. Here, we investigated the effects of vagus nerve stimulation (VNS) on poly-I:C-induced neuroinflammation and associated behavioral consequences in rats. Inflammatory cytokine IL-1β was induced in the prefrontal cortex of poly-I:C-injected animals and its level was decreaed by VNS. Production of phospho-NF-κB and phospho-IκB was increased by poly-I:C and downregulated by VNS, suggesting the involvement of NF-kB pathway in poly-I:C-induced neuroinflammation and regulation by VNS. Furthermore, phospho-Akt was downregulated by poly-I:C and strongly induced by VNS. Having noted increases in the number of activated microglial cells by poly-I:C in the prefrontal cortex, we investigated the effects of VNS on neuron-microglial interaction in poly-I:C-injected animals. Signals of chemokine receptor CX3CR1 bound to its ligand protein CX3CL1 were elevated by VNS in poly-I:C-injected animals, and pharmacological blockade of CX3CR1 activity inversely regulated the production of IL-1β, phospho-Akt and cleaved caspase 3 that was modulated by VNS. Behavioral assessments of pain and temperature sensation by von Frey and hot plate tests showed significant improvement by VNS, and forced swimming, marble burying and sucrose preference tests revealed that depressive-like, anxiety- and anhedonia-related behaviors caused by poly-I:C injection were significantly improved by VNS. Our data suggest that poly-I:C injection in rats induces neuroinflammation which is related to altered somatosensory and cognitive activities, and further implicate that VNS may be a useful therapeutic strategy to improve current treatments for neurological disorders.

Disclosures: U. Namgung: None. **K. Kim:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroinflammation in the EAE model of multiple sclerosis

Authors: *J. NEDDENS, M. DAURER, L. BREZNIK, R. RABL, T. LOEFFLER, S. PEINKIHER, S. FLUNKERT, S. SIDEROMENOS, M. PROKESCH;
Neuropharm., QPS Austria GmbH, Grambach, Austria

Abstract: Introduction:
Multiple sclerosis (MS) represents one of the most common neurological disorders among young adults. Key hallmarks of MS are demyelination, neuroinflammation, and neurodegeneration resulting in persistent invalidity. Experimental autoimmune encephalomyelitis (EAE) shows many pathological similarities to MS and is therefore often used as model to mimic MS by injecting myelin-oligodendrocyte-glycoprotein (MOG) in combination with pertussis toxin (PTX). Methods: C57Bl/6 mice were subcutaneously injected with MOG and 2 hours later intraperitoneally with PTX. One day later, animals were again injected with PTX. After another two weeks, animals were treated daily with the reference compound fingolimod or vehicle by oral gavage for 3 weeks. During the last treatment week, animals were tested for motor changes in the wire hanging and RotaRod test within one week and afterwards the spinal cord analyzed for pathological alterations such as myelination by Luxol fast blue staining and neuroinflammation by multichannel immunofluorescent labeling. Plasma of all animals was evaluated for Neurofilament light chain levels using the commercially available kit from UmanDiagnostics AB. Results: EAE-induced animals presented strong deficits in motor strength and motor behavior compared to vehicle-treated animals. Quantitative analysis of the spinal cord detected occurrence of focal demyelination accompanied by severe neuroinflammation as indicated by increased GFAP and Iba1 levels, presenting astrocytosis and activated microglia, respectively. Additionally, leukocytes and macrophages were strongly increased in the spinal cord as analyzed by CD45 and CD68 labeling, respectively. Neurofilament light chain as marker of neurodegeneration was severely increased in EAE-induced animals. Fingolimod was able to partially prevent the observed pathologies. Conclusions: Our results show that EAE induction causes the development of MS pathology 5 weeks after MOG treatment. Animals present a reduced muscle strength resulting in motor deficits as well as strong neuroinflammation and neurodegeneration. The EAE model thus mimics the key phenotypic and pathological hallmarks of multiple sclerosis and can be used to test new compounds against the observed pathologies. Fingolimod can be used as reference compound.

Disclosures: **J. Neddens:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **M. Daurer:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **L. Breznik:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **R. Rabl:** A. Employment/Salary

(full or part-time):; QPS Austria GmbH. **T. Loeffler:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Peinkiher:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Flunkert:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Sideromenos:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **M. Prokesch:** A. Employment/Salary (full or part-time):; QPS Austria GmbH.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Multiple Sclerosis Society RG 1807-32053

Title: Peripheral influenza A virus infection promotes T cell encephalitogencity and exacerbates relapsing experimental autoimmune encephalomyelitis in SJL/J mice.

Authors: *K. SOTO DIAZ[1], J. S. KIM[2], A. DAS[1,2,3], A. J. STEELMAN[1,2,4,5];
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Abstract: Multiple sclerosis is an inflammatory, demyelinating disease of the CNS in which most patients initially present with a relapsing-remitting form. Previous evidence suggests that respiratory viral infections increase the risk of relapse, but the mechanism remains unclear. The aim of this study was to elucidate the effects of influenza viral infection on disease progression in a mouse model of relapsing experimental autoimmune encephalomyelitis (R-EAE). Here, R-EAE was induced in female SJL/J mice then, two days after reaching peak disease, mice were inoculated with either sterile PBS or mouse-adapted human influenza A virus (IAV). Weights and scores were recorded daily. Mice were euthanized at day 16 post-IAV (p.IAV) (relapse) and day 24 p.IAV (post-relapse). At both time-points, antigen recall response assays were performed on lymphocytes from the spleen. Flow cytometry was used to determine whether infection altered immune cell profiles in brain, spinal cord, spleen, cervical lymph nodes, meninges and choroid plexus at day 16 p.IAV. To assess the effects of IAV on T cell polarizing cytokines, antigen presenting cells (APCs) were challenged with saline or increasing concentrations of IAV and cytokines measured from supernatants by ELISA. To determine if infected APCs altered the generation of specific Th cell subsets, naïve T cells from myelin-specific T cell receptor transgenic mice were cultured with saline or IAV pulsed APCs in the presence of their cognate antigen MOG_{33-55} for two days then the percentage of IFN-γ, GM-CSF and IL-17A producing cells determined by flow cytometry. Infection increased the number of relapses and worsened disease symptoms. Disease exacerbation in infected mice was associated with increased infiltration of myeloid cells in the brain and meninges compared to saline inoculated controls. Interestingly, the number of total T helper (Th) cells as well as IFN-γ and GM-CSF producing

Th cells was increased in the brain of infected mice compared to controls. Infection did not alter the number of IL-17A$^+$ Th cells. Similarly, splenocytes from infected mice produced more IFN-γ and GM-CSF, but not L-17A, than splenocytes from saline inoculated controls after stimulated with $PLP_{139-151}$. Notably, in response to IAV challenge, APCs secreted IL-12(p70) and IL-1β, but neither IL-6 nor IL-23. Finally, co-culturing T cells with IAV pulsed APCs and antigen increased the percentage of GM-CSF$^+$ cells compared to those cultured with control APCs and antigen. Taken together, these data suggest that infection exacerbates R-EAE, in part, by increasing T cell encephalitogenicity in the periphery and by promoting infiltration of immune cells into the CNS.

Disclosures: K. Soto Diaz: None. **J.S. Kim:** None. **A. Das:** None. **A.J. Steelman:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Peripheral immune challenges elicit differential up-regulation of hippocampal cytokine and chemokine mRNA expression in a mouse model of the 15q13.3 microdeletion syndrome

Authors: K. A. REES[1], K. M. MCCAMY[1], **U. H. WINZER-SERHAN**[2];
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Abstract: The human heterozygous 15q13.3 microdeletion is associated with neuropathological disorders, most prominently with epilepsy and intellectual disability. The 1.5 Mb deletion encompasses six genes (*FAN1* [*MTMR15*], *MTMR10*, *TRPM1*, *KLF13*, *OTUD7A*, and *CHRNA7*); all but one (*TRPM1*) are expressed in the brain. The 15q13.3 microdeletion causes highly variable neurological symptoms, and confounding factors may contribute to a more severe phenotype. *CHRNA7* and *KLF13* are involved in immune system regulation and altered immune responses may contribute to neurological deficits. We used the Df[h15q13]/+ transgenic mouse model with a heterozygous deletion of the orthologous region (Het) to test the hypothesis that the microdeletion increases innate immune responses compared to wild type (WT). Male and female mice were acutely challenged with the bacteriomimetic lipopolysaccharide (LPS, 0.1 mg/kg, i.p.) or the viral mimetic polyinosinic:polycytidylic acid (Poly(I:C), 5 mg/kg). Hippocampal mRNA expression of pro-inflammatory cytokines and chemokines were determined three hours after injection using quantitative PCR analysis. In controls, expression was not affected by sex or genotype. LPS and Poly(I:C) resulted in significantly increased hippocampal expression of cytokines, chemokines, and interferon-gamma (IFNgamma), with more robust increases for TNF-alpha, IL-6, IL-1beta, CXCL1, and CCL2 by LPS, higher induction of IFNgamma by Poly(I:C), and similar increases of CCL4 and CCL5 by both agents. Generally, Hets exhibited stronger responses than WT mice, and significant effects of genotype or genotype x treatment interactions were detected for CXCL1 and CCL5, and IL-6, IL-1beta, and CCL4, respectively,

after LPS. Sex differences were detected for some targets. LPS but not Poly(I:C), reduced overnight burrowing independent of sex or genotype, suggesting that LPS induced sickness behavior. Thus, mice carrying the microdeletion have an increased innate immune response following a LPS challenge, but further studies will have to determine the extent and mechanisms of altered immune activation and subsequent contributions to 15q13.3 microdeletion associated deficits.

Disclosures: K.A. Rees: None. **K.M. McCamy:** None. **U.H. Winzer-Serhan:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH, NIA R21AG072327

Title: Characterizing the accumulation of senescent-like p21+ myeloid cells in a mouse model of multiple sclerosis

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Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by a loss of myelin and axonal degeneration. The pathological outcome of MS is mainly known to be driven by both adaptive and innate immune cells. Early-stage MS is mediated by autoreactive T and B cells, while the later stage of the disease is mediated by activated microglia and macrophages resulting in chronic demyelination and neurodegeneration. However, the existing disease-modifying treatments that are effective early in MS have limited efficacy for late-stage MS. Therefore, there is an unmet need for therapies targeting late-stage MS. It has been suggested that the chronic low-grade inflammation in late-stage MS may result in inflammaging and the accumulation of the senescent cells. Cellular senescence, a hallmark of aging, is defined by a permanent cell cycle arrest induced by various stressors such as inflammation and are shown to be damaging to the tissue microenvironment through a senescent-associated secretory phenotype (SASP). However, to date, there have been limited studies providing evidence of cellular senescence and their potential role in MS pathogenesis. Here, we performed, MOG-induced experimental autoimmune encephalomyelitis (EAE) in mice, a preclinical model of MS, and observed a significant increase in senescence markers, p21 and p16, in the spinal cord at 28 days post-immunization (dpi) compared to naïve mice. Using immunostaining, we further show that while some of these cells are present in the lesion, the majority are localized to the leptomeninges adjacent to the ventral spinal cord demyelinating lesions. Moreover, we found that cells exhibiting senescence markers also co-expressed

lymphoid (B220) and myeloid (CD11b) lineage markers in the spleen and spinal cord of mice with EAE. Together, our findings demonstrate senescent-like immune cells in EAE accumulate in the meningeal compartment may contribute to neuroinflammation and demyelination. This further suggest that pharmacological clearance of senescent cells using anti-inflammaging or senolytic therapy may be beneficial in preventing or delaying disease progression.

Disclosures: Z. Manavi: None. **P. Gross:** None. **M. Cozart:** None. **J. Hu:** None. **M. Baydyuk:** None. **J.K. Huang:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: endMS doctoral studentship

Title: Oral ketone esters combined with metformin administration lead to better EAE clinical scores

Authors: *N. ALAEIILKHCHI[1], O. SEIRA[2], W. ZHOU[3], N. JANGJOO GHALAT[3], L. ZHANG[3], W. TETZLAFF[4];
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Abstract: Multiple sclerosis (MS) is an autoimmune-mediated demyelinating and neurodegenerative disease of the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) models the T cell-mediated demyelination in the CNS and has proven to be a useful preclinical model for translation of treatments in MS. Dietary treatments like ketogenic diet (KD) have shown promise in preclinical models and clinical MS. Adherence to KD is not desirable for most people with MS, especially in the long term. We think some of the benefits of the KD are because of the hepatic production of ketone bodies; therefore, we are testing exogenously provided ketone bodies in the form of ketone esters (KE) while EAE mice are on a nonketogenic diet to test that. Finally, we are combining KE with metformin, an antidiabetic agent which has shown great promise in people with MS. We hypothesize that KE by itself will lead to some behavioural recovery in mice models of MS but when combined with metformin the beneficial effects will be additively or synergistically augmented. We used the MOG 35-55 model of MS and had groups receiving 1. Control diet 2. Control diet + KE 3. Control diet + metformin 4. Control diet + KE + metformin. Our results show that combining KE with metformin had the highest efficacy in preventing the development of EAE signs, bringing down the disease scores. The thoracolumbar regions of the spinal cords are currently being analyzed for markers of oligodendrocyte regeneration, myelin preservation, and inflammation.

Disclosures: **N. Alaeiilkhchi:** None. **O. Seira:** None. **W. Zhou:** None. **N. Jangjoo Ghalat:** None. **L. Zhang:** None. **W. Tetzlaff:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Italian Ministry of Health, LR1

Title: Massive complement deposition and lymphocyte infiltration in the CNS of a model of epileptogenic cortical dysplasia

Authors: *F. COLCIAGHI[1], M. COSTANZA[2], A. CONSONNI[3], A. CIOTTI[1], B. CIPELLETTI[1], C. CAGNOLI[1], F. BAGGI[3], R. MANTEGAZZA[3], M. DE CURTIS[1]; [1]Epilepsy Unit, [2]Mol. Neuro-Oncology Unit, [3]Neuroimmunology and Neuromuscular Disorders Unit, Fondazione IRCCS Inst. Neurologico Carlo Besta, Milano, Italy

Abstract: Blood-brain barrier (BBB) damage and the activation of innate and adaptive immune components have been implicated in the generation of acute seizures and, in some circumstances, in the development of chronic epilepsy in both experimental models and patients. The complement system or cascade (ComC) is a key innate immune mechanism encompassing soluble and cell membrane proteins which, through three different pathways, culminates in membrane attack complex (MAC) assembly on the cell surface and death of the target cell. The overexpression of various ComC products has been found in surgical tissues from patients with temporal lobe epilepsy (TLE) and with focal cortical dysplasia (FCD), as well as in several epilepsy models. However, how ComC gets activated in the epileptogenic brain and how this impacts tissue integrity has not been fully elucidated yet. To this aim, we exploited the established MAM/pilocarpine (MP) rat model of drug-resistant epilepsy associated with CD, a cause of refractory epilepsy in patients, and verified whether the occurrence of experimental status epilepticus (SE) could induce ComC activation and consequent MAC deposition in the epileptic brain. MP rats were sacrificed 18h after SE (MP-acute) or 4 weeks after the onset of spontaneous seizures (MP-chronic) and compared to non-epileptic MAM control rats (at least n=10 rats/group). IF/WB analyses revealed a progressive increase of IgG extravasation (MP-chr vs MAM $p<0.001$; MP-chr vs MP-ac $p<0.01$), and destructive IgG-C1q and IgG-MAC deposition on neurons. The formation of C1q-IgG complex was confirmed by co-immunoprecipitation analysis of leaked IgG isolated from cortical homogenates (MP chr vs MAM $p<0.001$). Furthermore, qRT-PCR analysis of ComC factors revealed a progressive and significant increase in the expression levels of ComC factors belonging either to the classical or alternative pathways (C1q, C1R, C3, C5, C7, C8, FB and FD) in the hippocampus of MP-chr vs MP-18h vs MAM rats, indicating that the epileptogenic brain per se can sustain intra-cerebral complement synthesis. Interestingly, pilocarpine-induced SE in MAM rats prompted

accumulating gliosis and severe CD20+ and CD8+ lymphocytes infiltration in MP rat brains (MP-ac vs MAM $p<0.01$; MP-chr vs MAM $p<0.001$). Data here reported indicate that the epileptogenic MAM/pilocarpine rat brain is characterized by strong neuroinflammation, encompassing both innate and adaptive immune mechanisms, suggestive of encephalitis-like features, and indicate that complement-dependent cytotoxicity may represent a key effector mechanism in the experimental epileptogenic brain.

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Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Mouse neuroinflammation models

Authors: M. S. HEINS, I. M. VUIST, M. F. OLTHUIS, *G. FLIK;
Charles River Labs., Groningen, Netherlands

Abstract: Neuroinflammation is a process implicated in many neurodegenerative disorders. The blood-brain-barrier (BBB) ensures an immune privileged state of the brain, rendering a peripherally induced inflammation less suitable for investigation of the neuroinflammatory process. Since the peripheral inflammation may cross over to the CNS by impacting the integrity of the BBB and thereby can deliver inflammatory cells and cytokines from the bloodstream to the brain. A more representative model would thus demonstrate local neuroinflammation with preferably no peripheral effects.
Here, we investigated the optimal experimental design for a locally induced neuroinflammation model using several stimuli (LPS, BzATP and LPS+BzATP) in adult male C57Bl/6J mice. The pro-inflammatory cytokine levels in several tissues and fluids, including interstitial fluid (ISF), collected either by push pull microdialysis or open flow microperfusion, blood and brain tissues were evaluated. Specific attention was paid to the differential distribution and localization of the pro-inflammatory cytokines following the different stimuli.
We aim to demonstrate the effects of the tested treatments on neuroinflammation as well as the potential differences in peripheral effects of the tested set ups. Combined data should present us with guidelines for choosing optimal experimental designs for a variety of neuroinflammatory research questions. In addition, we will gain a better understanding of the timing effects of priming and activation of the inflammasome. Allowing better decisions to be made on test article administration for the various set ups.

Disclosures: M.S. Heins: None. **I.M. Vuist:** None. **M.F. Olthuis:** None. **G. Flik:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI 20K07958

Title: Preconditioning of Lipopolysaccharide improves anxiety-like behavior and brain inflammation associated with chronic corticosterone exposure in mice

Authors: R. NAKAGAWA[1], ***H. TODA**[1], M. KOGA[1], M. SATO[1], M. NAGAMINE[2], S. ASAI[1], A. YOSHINO[1];
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Abstract: Microglia play an important role in neuroinflammation and have recently attracted attention as a target for depression treatment. Previously, prior low-dose administration of lipopolysaccharide (LPS preconditioning) inhibited microglial activation by subsequent high-dose LPS administration, suggesting a potential preventive effect on the onset of depression. However, its impact on models other than the high-dose LPS-induced systemic inflammation depressive model was unknown. Therefore, we investigated whether LPS preconditioning is effective in a chronic corticosterone exposure model, one of the well-known pathophysiology models in depression, to clarify the mechanism of action of LPS preconditioning and its potential application in preventive treatment. C57BL/6 mice (6wk-old, male) were administered with corticosterone via drinking water, including 140 μg/ml for four weeks; the LPS preconditioning group received an intraperitoneal injection of LPS at 0.2 mg/kg 8 and 7 days prior corticosterone administration. Mice were assigned into three groups; a control group, a corticosterone group (COR) and a corticosterone+LPS preconditioning group (COR+LPS). After four weeks of corticosterone administration, an open field test and a marble-burying test were conducted. After these behavioral tests, brains were extracted and dissected into the hippocampus. Then, gene expression analysis was performed on the hippocampus by real-time PCR. As a result of behavioral test, COR group showed increased anxiety-like behavior, however not in the COR+LPS group. In the mRNA expression analysis, expression in *Il-1β*, *Nlrp3*, *Asc*, *Myd88*, and *Tlr4* genes increased in COR group, however not in COR+LPS. Especially, *Il-1β*, *Nlrp3*, *Asc*, and *Myd88* genes indicated a significant decrease in COR+LPS compared to COR. The present study suggested an inhibitory effect of LPS preconditioning on neuroinflammation induced by chronic corticosterone exposure. On the other hand, the effect of LPS preconditioning on suppressing microglial activation was not indicated in the chronic corticosterone exposure model, suggesting that LPS preconditioning suppresses inflammation and anxiety-like behavior by inhibiting the TLR4-NLRP3 pathway. LPS preconditioning has potential applications in the preventive treatment of depression, a sterile inflammation.

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Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Following changes in brain structure and function with multimodal MRI in a year-long prospective study on the development of Type 2 diabetes

Authors: *Y. WANG[1], N. RUBALCABA[1], A. GHAW[1], S. BALAJI[1], Y. KWON[1], C. MUNSON[1], M. POMPILUS[1], M. FEBO[1,2], P. KULKARNI[1], C. FERRIS[1,2];
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Abstract: The development of Type-2 diabetes (T2DB) is a devastating disease affecting over 462 million people worldwide. To our knowledge there has never been a prospective study following disease progression from a normal brain to fulminating diabetes in the same subject. To that end, we used non-invasive multimodal MRI to follow changes in brain microstructure and function for one year in rats developing T2DB. The developing neuropathology was correlated with measures of cognitive and motor function. Normal, healthy male rats ca 100 days of age were treated with streptozotocin and put on a high fat, high fructose diet, an established model for T2DB. At 3,6-, 9- and 12-month intervals rats destined for T2DB and age matched controls on a normal diet were studied for changes in brain structure using voxel-based morphometry, alteration in white and gray matter microarchitecture using diffusion weighted imaging and functional connectomics. Images from each modality were registered to, and analyzed, using a 3D MRI rat atlas providing site-specific data on over 171 different brain areas. Weight gain and loss of glucose tolerance were obvious at 3 months. By 9-12 months there were significant changes in brain morphology and microarchitecture highlighted by a reduction in brain volume, alterations in gray matter architecture, and decrease in functional coupling with disease progression. Significant changes in motor and cognitive behavior and tolerance to pain were not realized until 12 months. There was a dramatic decrease in brain volume by 6 months in T2DB rats. A significant increase in indices of fractional anisotropy in cerebellum, brainstem, midbrain dopaminergic area, and prefrontal cortex were present by 9 months, a possible consequence of reduced brain volume.

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Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: U54 AG054345

Title: Targeting the immune contributions to neurodegeneration via the IL1RAP signaling axis

Authors: *K. P. KOTREDES[1], G. A. PRIETO[3], M. SASNER[2], G. W. CARTER[2], C. W. COTMAN[4], G. R. HOWELL[2];
[1]The Jackson Lab., [2]The Jackson Lab., Bar Harbor, ME; [3]Inst. de Neurobiología, Juriquilla, Mexico; [4]UC Irvine, Irvine, CA

Abstract: With the evolution of next-generation-sequencing technologies the availability of human disease reference datasets is growing. From these resources genetic loci and risk factors are emerging, correlated to the prevalence or magnitude of disease burden. With this information we have found that neurodegenerative disorders are linked to specific immune processes. Evidence ties synapse-loss, cognition, amyloid burden, and cytokine production to immune genes, microglia, infection, and aging. Recently the *IL1RAP* gene was found to be a strong risk factor for amyloid accumulation in Alzheimer's disease (AD) patients. This association was strengthened in patients that also expressed *APOEε4*, a well-described late-onset AD (LOAD) risk factor. However, mouse models of LOAD are in short-supply with the most utilized strains simply expressing amyloid-related, familial AD transgenes and failing to address the metabolic, immune, and homeostatic processes we know to be dysregulated in human disease. To meet this need we have created three new mouse strains to investigate this risk factor. IL1RAP is a membrane-bound IL-1ß receptor expressed in most tissues, including CNS-specific astrocytes, microglia, and neurons. It regulates immune signaling via downstream gene expression through NF-κB and MAP kinase transcription factors. Further, a neuron-specific splice variant of *IL1RAP*, *IL1RAPb*, has been identified and appears to inhibit cytoplasmic signaling by preventing binding to MYD88. As a suspected regulatory mechanism for IL-1ß vulnerability, IL1RAP/IL1RAPb may therefore prove to be a potential target for therapeutic intervention against neuron loss. Initial studies in an aged mouse model with a LOAD-relevant genetic background ('B6.LOAD1' expressing *APOEε4* and *Trem2*R47H* alleles) showed that 'knocking-in' a ubiquitous *Il1rap* exon 2 deletion produced a transcriptional signature in the brain more similar to those seen in AD patients, supporting observations from human datasets. To exacerbate this phenotype, we developed an additional strain employing a full knock-out of systemic *Il1rap* transcript on a 'LOAD2' background (*APOEε4, Trem2*R47H, and hAPP)*. For a closer investigation into how neuronal *Il1rap* is functioning exclusively, we also generated an *Il1rapb*-specific inducible knock-out model that allows expression control of this transcript and, ideally, intensity of subsequent inflammation. Accordingly, we are suited to investigate many aspects of IL1RAP function layered with *APOEε4* and amyloid. Characterization of these novel mouse strains are ongoing and experimental cohorts are aging prior to further experimentation.

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Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

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Program #/Poster #: 624.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA Merit 1I01BX005180

Title: Abnormal T cell homeostasis in the motor cortex of $SOD1^{G93A}$ mice

Authors: *B. MOLLIGODA[1,2], Y. JUNG[1,2,4], C. M. TOGNONI[1,2], I. CARRERAS[1,2,3], A. DEDEOGLU[1,2,5];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by muscle atrophy and degeneration of motor neurons in the central nervous system (CNS). This degeneration occurs alongside a confluence of non-cell autonomous changes, including potent inflammatory changes mediated by infiltrating T cells. Prior research has suggested that certain subpopulations of T cells play separate roles in ALS pathophysiology, with $CD8^+$ T cells promoting neuroinflammation and $CD4^+$ T cells such as T regulatory (Treg) and T helper 2 (Th2) cells holding it in check. Despite the distinct contributions of these T cell subpopulations to the inflammatory response, it is not well understood how the balance of $CD4^+$ and $CD8^+$ T cells is altered in the CNS during ALS pathoprogression. The present study sought to examine potential changes in these T cell subtypes in the motor cortex of transgenic $SOD1^{G93A}$ mice, a mouse model of ALS, at three pathologically relevant stages: the pre-symptomatic stage at 30 days of age, the onset stage at 95 days of age, and the end stage at 120 days of age. At the specified ages, $SOD1^{G93A}$ and wildtype (WT) littermates were euthanized, and brains were collected for analysis. To ensure validity, all subsequent analysis was performed blinded to genotype. Sets of brain sections were analyzed by immunohistochemistry using antibodies to detect $CD4^+$ and $CD8^+$ T cells. We observed increased numbers of $CD8^+$ T cells and decreased numbers of $CD4^+$ T cells in $SOD1^{G93A}$ mice compared to WT littermates, reaching significance for $CD8^+$ T cells at the 120-day old end stage. The ratio of $CD4^+$ to $CD8^+$ T cells, an important composite measure of the balance between these T cell subpopulations, was also found to be significantly lower in 120-day old $SOD1^{G93A}$ mice compared to WT mice. To further analyze T cell-related changes, we measured the gene expression of FoxP3 and GATA-3, transcription factors required for the differentiation of precursor $CD4^+$ T cells into either Tregs or Th2 cells, respectively, by quantitative real-time PCR (QRT-PCR) analysis of RNA samples from the motor cortex of these mice. We found that the level of FoxP3 gene expression increased

significantly in WT mice from 30 to 120 days of age but such increase was not detected in $SOD1^{G93A}$ mice. Similarly, while GATA-3 gene expression increased in WT mice with age, its gene expression was suppressed in $SOD1^{G93A}$ mice. These results emphasize the loss of T cell homeostasis during the development of motor neuron dysfunction in ALS.

Disclosures: B. Molligoda: None. **Y. Jung:** None. **C.M. Tognoni:** None. **I. Carreras:** None. **A. Dedeoglu:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FISM Grant 2017/R/11

Title: The role of meningeal neural progenitors in brain auto-reactive immune cell regulation

Authors: *I. DECIMO[1], F. CIARPELLA[1], S. ZORZIN[1], C. L. MARTIN[4], A. BANI[2], B. ROSSI[2], S. DUSI[2], A. CORSI[3], G. F. FUMAGALLI[1], B. M. DOS SANTOS LIMA[3], S. DOLCI[1], N. PIAZZA[1], P. TORTELLA[5], N. LOPEZ[2], A. AMENTA[6], L. SCHIRMER[4], G. CONSTANTIN[2], F. BIFARI[6];
[1]Dept. of Diagnostics and Publ. Health, Sect. of Pharmacol., [2]Dept. of Medicine, Sect. of Gen. Pathology, [3]Section of Gen. Pathology, Dept. of Med., Univ. of Verona, Verona, Italy; [4]Heidelberg Univ., Heidelberg, Germany; [5]Fac. of human and social science, Univ. of Enna Kore, Enna, Italy; [6]Dept. of Med. Biotech. and Translational Med., Univ. of Milan, Milan, Italy

Abstract: Meninges represent a checkpoint at which activated brain-reactive T cells are licensed to enter the Central Nervous System (CNS) parenchyma and subsequently to damage neural tissue. Beside this role, meninges have emerged as more complex structure able to modulate CNS morphogenesis and function. Meninges are heterogeneous tissue composed by both immune and stromal cells including pericytes, arachnoidal fibroblasts, phagocytes and lymphatic vessels. We have identified a neural progenitor cell (NPC) population present in meningeal substructures that migrate to the cortex and differentiate into functional integrated neurons. Notably, meninges have been shown to react following injury, increasing the NPC and blood-born immune cell populations. However, the role of meninges and their contribution to brain disease is still poorly explored. We aimed to deepen the role of meningeal structures and meningeal stem cell niche in the progression of Multiple Sclerosis (MS), a chronic inflammatory neurodegenerative disease of the CNS. We established an animal model of experimental autoimmune encephalomyelitis (EAE), and we characterized the meningeal NPCs at different stages of the pathology by combining immunofluorescence and single cells transcriptomic analysis. We found a modification in number and distribution of NPCs, stromal and immune cells within mice brain meninges during EAE progression. In particular, we observed a robust

EAE-induced accumulation of cells in the meningeal substructure underneath the hippocampus, with local parenchymal infiltration. In line with this observation, we found increase demyelination and reactive gliosis in the hippocampal region of EAE mice brain. Single cell RNAseq further characterized the cell populations and the stromal-immune cell interactions of EAE mice brain meninges. With this study we, for the first time, highlighted the role of stromal meningeal cells in the pathogenesis and progression of EAE. These results may uncover novel cellular and molecular mechanisms that might be potential pharmacological targets for treatments of MS.

Disclosures: **I. Decimo:** None. **F. Ciarpella:** None. **S. Zorzin:** None. **C.L. Martin:** None. **A. Bani:** None. **B. Rossi:** None. **S. Dusi:** None. **A. Corsi:** None. **G.F. Fumagalli:** None. **B.M. Dos Santos Lima:** None. **S. Dolci:** None. **N. Piazza:** None. **P. Tortella:** None. **N. Lopez:** None. **A. Amenta:** None. **L. Schirmer:** None. **G. Constantin:** None. **F. Bifari:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AI144004
NIH Grant NS112727
NMSS (US) Grant RG-1807-31964
NMSS (US) Grant RG-1508-05912

Title: Docosahexaenoic acid-derived pro-resolving lipid mediator maresin-1 ameliorates inflammation and prevents disease progression in preclinical model of multiple sclerosis

Authors: *I. ZAHOOR[1], M. NEMATULLAH[1], S. MIR[1], J. WATERS[1], I. DATTA[2], M. CERGHET[1], L. M POISSON[2], R. RATTAN[3], S. GIRI[1];
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Abstract: Multiple sclerosis (MS) is one of the most common inflammatory and neurodegenerative diseases in young adults leading to a build-up of neurological defects with an irreversible disability. Unresolved inflammation represents its pathological hallmark; however current therapeutic options fail to adequately suppress the ongoing inflammation, resulting in chronic inflammation. Studies suggest that the endogenous mechanisms to resolve inflammation are intact but become defective in patients due to deficiency of downstream resolution mediators, thus resulting in disease progression and continued neuronal damage. Docosahexaenoic acid (DHA) metabolism being defective in MS, we hypothesize that supplementation of its downstream metabolite, maresin 1 (MaR1) will resolve inflammation and demyelination in its preclinical animal model, experimental allergic encephalomyelitis (EAE). We performed a comparative metabolite profiling using targeted metabolipidomics in serum samples from 29

relapsing-remitting (RRMS) patients and 29 age and gender-matched healthy controls (HC). For therapeutic effect of MaR1, we induced EAE in SJL mice, followed by intraperitoneal treatment with 300ng of MaR1 from day1 post-disease induction. We evaluated the effect on disease severity and inflammation by monitoring disease course of EAE, recall response by ELISA, cytokine expression analysis by qPCR and western blotting, and immune profiling by flow cytometry. Also, the neuroprotective effect of MaR1 through myelination was assessed by single molecule array (SIMOA) assay and histopathology. Statistical analysis was done using Graph-Pad Prism. Metabolite profiling revealed significant imbalance ($p<0.05$) between inflammatory response and resolution process in MS, confirming the metabolic dysfunction of resolution mediators including MaR1. Restoration of MaR1 prevented disease progression and reduced disease severity in EAE by inhibiting the infiltration of immune cells (CD4+IL17+ and CD4+FNγ+) in CNS as shown by intracellular staining ($P<0.001$). Recall response showed that MaR1 significantly inhibited pro-inflammatory cytokine IL17 ($P<0.01$) and promoted IL10 and IL4 production ($P<0.001$). Also, it exerted neuroprotective effects as we found lower levels of NFL ($P<0.01$) in the plasma of treated mice compared to control, which was further confirmed by higher expression of MBP in brain from MaR1 treated group. Overall, our targeted metabolipidomics in MS patients identified MaR1 deficiency, whose supplementation exerts anti-inflammatory and neuroprotective effects in preclinical animal model, suggesting MaR1 could be a new therapeutic molecule in MS.

Disclosures: I. Zahoor: None. **M. Nematullah:** None. **S. Mir:** None. **J. Waters:** None. **I. Datta:** None. **M. Cerghet:** None. **L. M Poisson:** None. **R. Rattan:** None. **S. Giri:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 HD097990, to VJ-T
NIH Grant R01 GM118197, to VJ-T
NIH Grant F32 HD101357, to OHC
CU Medicine Endowment to VJ-T

Title: Systemic inflammation exacerbates sevoflurane-induced developmental neurotoxicity in rat neonates

Authors: *N. USEINOVIC[1], S. MAKSIMOVIC[1], C. LIECHTY[1], O. H. CABRERA[1], N. QUILLINAN[1,2], V. JEVTOVIC-TODOROVIC[1,3];
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Abstract: Mounting preclinical and clinical evidence suggests that general anesthesia administered during the neonatal period induces acute neuronal apoptosis and long-term behavioral deficits. Although traditionally studied in isolation, the clinical reality is that surgical scenarios which necessitate anesthesia in the first place are often accompanied by systemic inflammatory response. The impact of underlying inflammation in the context of anesthesia-induced developmental neurotoxicity remains largely unknown. To address this question, Sprague-Dawley rat pups at postnatal day 7 (PND7) were randomly assigned to receive either sevoflurane (3% for 3h) or carrier gas 12h after lipopolysaccharide (LPS, 1 μg/g) or vehicle injection. To investigate mechanistic pathways of neuronal injury, a separate cohort of animals was additionally pretreated with Vx-765, a pharmacological inhibitor of caspase-1. Acute injury and changes in hippocampal interleukin levels were quantified 2h after the end of anesthesia. Long-term functional outcomes were assessed using a battery of behavioral tests in memory and anxiety domains at 5-8 weeks of age. Histomorphological quantification of activated caspase-3 and -9 revealed significant upregulation in the subiculum and CA1 hippocampal regions after either sevoflurane or LPS treatments, which was further exacerbated when sevoflurane was administered in the settings of underlying LPS-induced inflammation. The acute neuronal injury induced by LPS+sevoflurane led to significant, sex-specific behavioral deficits when tested at 5-8 weeks, with learning and memory deficits in males and heightened anxiety-related behavior predominantly affecting female rats. Both LPS and LPS+sevoflurane treatments resulted in upregulation of caspase-1 and NLRP1, but not NLRP3, along with related proinflammatory interleukins IL-1β and IL-18. Selective inhibition of caspase-1 by Vx-765 downregulated IL-1β hippocampal levels in LPS and LPS+sevoflurane groups. Furthermore, Vx-765 pretreatment effectively decreased caspase-9 and -3 immunoreactivity compared to vehicle controls. Taken together, these findings indicate that systemic inflammation plays an important, yet often overlooked, role in promoting neuronal injury induced by neonatal sevoflurane administration. The long-term consequences of sevoflurane-induced, inflammation-propagated neuronal injury present as sex-specific deficits in either learning and memory, or the anxiety-related domain. Finally, our experiments with Vx-765 indicate that these deficits are mediated, at least in part, by the activation of caspase-1/-9/-3 axis in settings of inflammation.

Disclosures: N. Useinovic: None. **S. Maksimovic:** None. **C. Liechty:** None. **O.H. Cabrera:** None. **N. Quillinan:** None. **V. Jevtovic-Todorovic:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.18

Title: WITHDRAWN

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R21NS121642

Title: In vivo mapping multiple mouse embryos with manganese-enhanced magnetic resonance imaging (memri)

Authors: *B. ZHANG, Y. JIANG, J. MANDEVILLE, S. KISLAL, A. EDLOW, X. YU; Massachusetts Gen. Hosp., CHARLESTOWN, MA

Abstract: Manganese-enhanced magnetic resonance imaging (MEMRI) can be used to enhance T1 contrast. However, we need an in vivo MRI imaging method to quantify paravascular clearance of hydrocephalic brain with high resolution and sampling rate. To trace the cerebrospinal fluid (CSF) flow dynamics in embryonic brains, we used a 3D modified driven equilibrium Fourier transform (3D-MDEFT) sequence with faster sampling rates based on the Mn-enhanced contrast in ventricles of embryonic brains. A 14T horizontal MR scanner was used for image acquisition. Embryonic day 17.5 mice were imaged after Mn injection through tail-vein (10mM, 0.1ml in saline). 25% Mannitol (0.1ml) was injected to break the blood placenta barrier 4-30 mins before Mn injection. Before Mn injection, a Rapid Acquisition with Relaxation Enhancement (RARE) sequence was acquired for anatomical reference (TE=2.5 ms; TR=2382 ms; segment factor=6; FOV= 28.8 x 28.8 x 24 mm^3; isotropic 150 μm^3). 3D MDEFT was performed to measure CSF flow (TE=2.5 ms; TR=2382 ms; segment factor=6; FOV= 28.8 x 28.8 x 24 mm^3; isotropic 150 μm^3). Here we show high-resolution 3D-MDEFT images with a higher contrast-to-noise ratio (CNR) than the previous *in utero* and postnatal MEMRI studies. Fig 1 shows multiple fetal brain mapping with both RARE image (Fig 1A) and 3D-MDEFT-based MEMRI (Fig 1B). 3D-MDEFT enables visualization of Mn clearance from ventricles in embryonic brains. Our data shows the Mn-enhanced fetal brain with MDEFT from three embryos. A 3D reconstruction to identify each fetal brain is shown in Fig 1C. After registering three embryos to the same template, we averaged the line profiles of Mn-enhanced MRI signals through the lateral ventricles, showing robust signal drop due to the Mn clearance from the ventricles (Fig 1D). This work demonstrates the feasibility to map Mn-enhanced MR signal in embryonic brains, achieving high CNR specific for Mn-based T1 signal as a potential indicator of chronic paravascular clearance of Mn from the parenchyma.

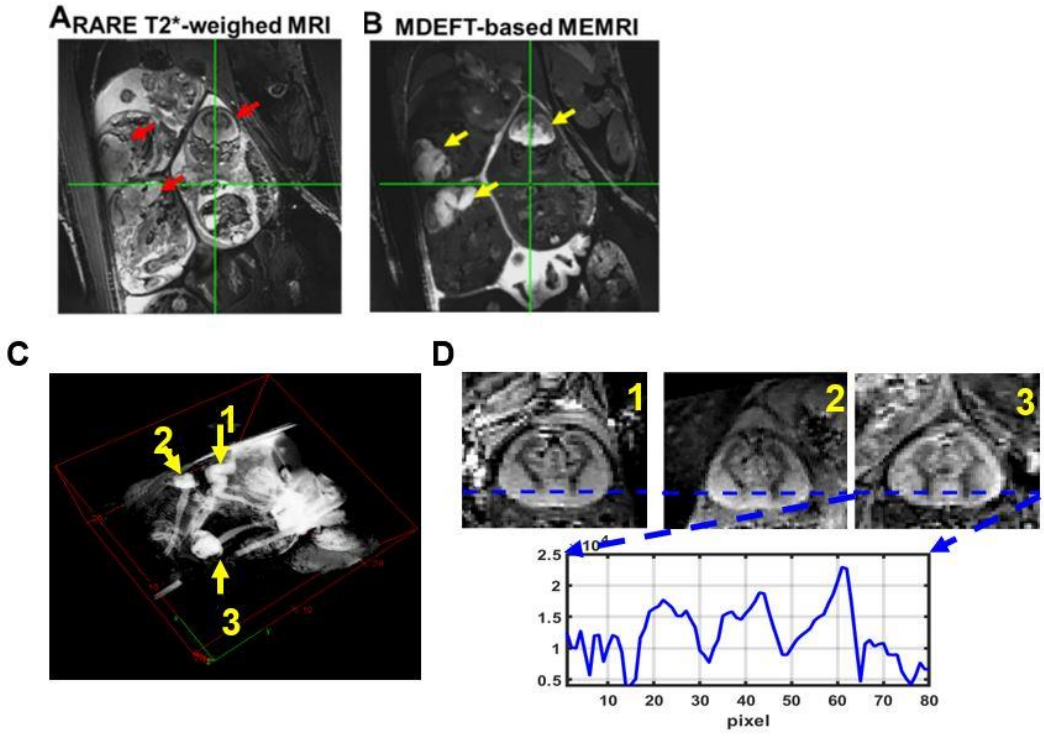

Figure 1. **A.** The T2*-weighed MRI imaging of three E18 embryos (red arrows). **B.** The 3D-MDEFT-based MEMRI to map the Mn-enhanced fetus brains (yellow arrows) **C**. A 3D reconstruction to identify each fetal brain (yellow arrows) in the sac based on the maximal density projection. **D**. The registered 3 embryos with an Mn-enhanced lie profile through ventricles in #3 embryo.

Disclosures: B. Zhang: None. **Y. Jiang:** None. **J. Mandeville:** None. **S. Kislal:** None. **A. Edlow:** None. **X. Yu:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Lupus Foundation Gina M Finzi Summer Fellowship Award

Title: Exploring the role of lipocalin-2 in neuropsychiatric lupus

Authors: *S. GARCIA, E. V. MIKE, C. PUTTERMAN;
Microbiology and Immunol., Albert Einstein Col. of Med., Bronx, NY

Abstract: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects multiple organ systems. Neuropsychiatric lupus, or NPSLE, refers to the poorly understood brain

manifestations of SLE. Patients experiencing NPSLE often suffer from depression and cognitive deficits, contributing to poorer prognosis and quality of life. Potential mechanisms important in NPSLE include blood brain barrier (BBB) disruption and localized neuroinflammation. Lipocalin-2 (LCN2) is a multi-functional acute phase protein known to affect immune cell function, BBB integrity, and glial cell activation. The MRL/lpr strain is a widely used mouse model for NPSLE, capturing multiple human facets of the disease, including the presence of anti-nuclear antibodies, kidney dysfunction, depressive-like behavior and cognitive deficits. We generated an LCN2-deficient MRL/lpr mouse (LCN2KO) to examine the effects of LCN2 deficiency on the behavioral deficits in murine lupus. We compared the neurobehavioral profile of 16 week old female MRL/lpr and age and sex matched LCN2KO mice. The mice were sacrificed promptly after behavioral tests and tissues were taken and fixed in paraformaldehyde for immunofluorescent staining. To evaluate systemic disease, we measured the presence of anti-nuclear antibodies, kidney disease, and serum IgG. We found that while LCN2 deficiency in MRL/lpr mice did not affect systemic lupus manifestations, behavioral analysis showed significant improvement in Porsolt forced swim and object placement tests (fig 1), suggesting a brain specific role of LCN2. To evaluate glial cell activation, we stained brain tissue from MRL/lpr and LCN2KO mice for GFAP (astrocytes) and Iba1 (microglia) and evaluated mean fluorescence intensity and morphology. We found a higher intensity of GFAP expression in hippocampal astrocytes in MRL/lpr compared to the LCN2KO mice. Our behavioral results indicate a potential role of LCN2 in perpetuating brain inflammation and hippocampal astrocyte activation, that subsequently can contribute towards depression and cognitive deficits in NPSLE.

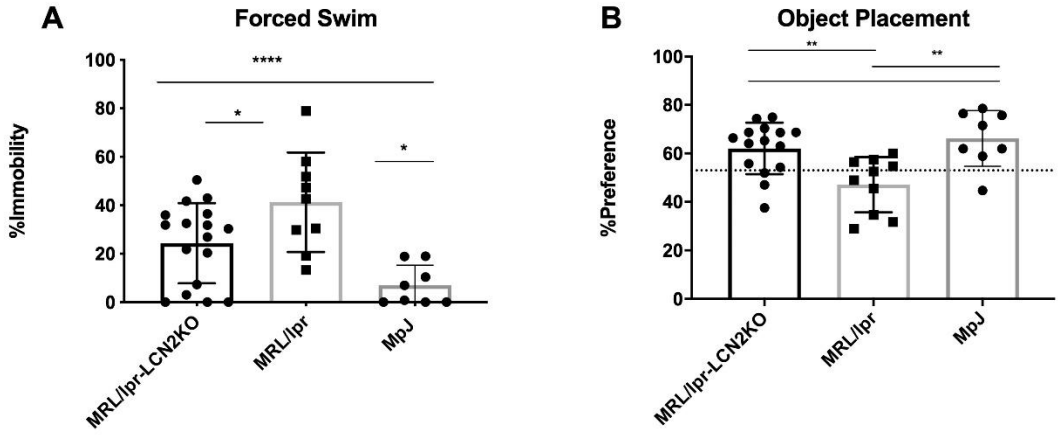

Figure 1: Behavioral assessment of LCN2 deficient MRL/lpr mice shows significant improvement in affective and cognitive deficits. We observed significant improvement in the Porsolt forced swim test, which evaluates depression (panel A) and the object placement test, which evaluates spatial memory (panel B)

Disclosures: S. Garcia: None. **E.V. Mike:** None. **C. Putterman:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DoD grant W81XWH1810374 awarded to Dr. S. Chatterjee.
VA Merit Award I01CX001923-01 awarded to Dr. S. Chatterjee.

Title: Prolonged acetaminophen exposure exacerbates neuroinflammation and neurodegeneration via a potential microbiome-liver-brain axis in Gulf War Illness symptom persistence mouse model

Authors: *D. BOSE[1], P. SAHA[1], R. SETH[1], S. ROY[1], A. TRIVEDI[1], M. MORE[1], S. CHATTERJEE[1,2];
[1]Univ. of South Carolina, Columbia, SC; [2]Columbia VA Med. Ctr., Columbia, SC

Abstract: Long term use of acetaminophen (APAP) among war Veterans for chronic pain management have been reported in earlier studies. Studies also suggested that prolonged APAP administration induced hepatotoxicity which further led to neuroinflammation. Gulf War Illness (GWI) is chronic multisymptomatic condition which continues to persist among a subsection of GW Veterans till now. It is characterized by musculoskeletal pain, neurological disorder and cognitive dysfunction among the major symptoms reported by the Veterans. Use of analgesics like APAP is prevalent among GW Veterans to alleviate the post war malaise. In this study we investigated whether prolonged APAP dosage exacerbates GW chemical induced neuroinflammation using an established GWI persistence model (5 months). Adult C57BL/6J mice was administered with GW chemicals pyridostigmine bromide (2mg/kg) and permethrin (200mg/kg) (n=6) and another group were exposed with GW chemicals along with APAP (300mg/kg) dosing for 1 month (n=6). Bacteriome analysis showed significant decrease in relative abundance of bacteria promoting brain homeostasis like *Lachnospiracae, Enterococcus faecium, Roseburia* and *Oscillibacter* in mice group co-exposed with GW chemicals and APAP compared to mice group exposed to GW chemicals only. Liver pathology study showed significantly increased Kupffer cell activation by increased CD68, F4/80 expression and increased expression of the proinflammatory cytokine IL-1β due to prolonged APAP dosage in GWI conditions. Serum lipocalin 2 and IL-6 levels, having a role in brain pathology, were significantly increased mice group treated with GW chemicals and APAP. Further, decrease in expression of tight junction protein claudin 5 and matrix metalloprotease 9 in the brain of mice group co-exposed with GW chemicals and APAP suggested blood brain barrier disruption. Increased astrocyte activation and expression of proinflammatory cytokines IL-6 and Il-1β in mice administered with GW chemicals and APAP indicated increased neuroinflammation. Decreased expression of synaptic plasticity marker brain-derived neurotrophic factor (BDNF) and increased argyrophilic structures by Bielschowsky staining suggested increased neurodegeneration due to prolonged APAP exposure in GWI condition. Finally, prolonged APAP dosage in GW conditions exacerbated neuroinflammation and neurodegeneration induced by GW chemicals via a potential microbiome-liver-brain axis.

Disclosures: D. Bose: None. **P. Saha:** None. **R. Seth:** None. **S. Roy:** None. **A. Trivedi:** None. **M. More:** None. **S. Chatterjee:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS122961

Title: Assessing the Maturation State of Infiltrating Neutrophils in the Acutely Injured Spinal Cord

Authors: *M. KIRCHHOFF[1], D. MCCREEDY[1,2];
[1]Biol., Texas A&M Univ., College Station, TX; [2]Texas A&M Inst. for Neurosci., College Station, TX

Abstract: Neutrophils are the first type of peripheral immune cell to rapidly infiltrate the spinal cord in large numbers after spinal cord injury (SCI); however, their role during the acute phase of injury remains unclear. While the biological functions of different neutrophil subtypes have been reported under homeostatic conditions, neutrophil heterogeneity remains poorly understood in the context of SCI. In this study, we characterized the maturation state of infiltrating neutrophils acutely after SCI. We used flow cytometry to measure the expression of CD101 on neutrophils isolated from spinal cord or blood collected 0-, 4-, and 24-hours post-injury (hpi). Neutrophils ($CD11b^{+}/Ly6G^{+}$ cells) with minimal expression of CD101 ($CD101^{lo\text{-}neg}$) were identified as immature neutrophils, and those with high expression of CD101 ($CD101^{hi}$) as mature. At the time of injury (0-hpi), the spinal cord contains very few neutrophils and similar to neutrophils in blood, they display a predominantly mature phenotype ($CD101^{hi}$). With time, neutrophils in the blood gradually shifted from mature to mostly immature neutrophils by 24-hpi, likely reflecting early release of immature neutrophils from the bone marrow into circulation. In contrast, neutrophils in the spinal cord were predominantly immature at 4-hpi and shifted to a more mature phenotype by 24-hpi. To further investigate the opposing trends in neutrophil maturity after SCI, we are using 5'-ethynyl-2'-deoxyuridine (EdU) and flow cytometry to differentially track neutrophils by maturation state in the blood, bone marrow, and spinal cord tissues. In future studies, we will apply these methods to better characterize the neutrophil subtypes that infiltrate the spinal cord during acute periods of SCI.

Disclosures: M. Kirchhoff: None. **D. McCreedy:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

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Program #/Poster #: 624.23

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: U.S. Prov. Appl. No. 63/318,612
PCT/US21/52964

Title: Development and characterization of a microglia-restricted mouse model of NLRP3 driven neuroinflammation

Authors: *E. CARLONI[1], B. E. RANKIN[1], A. L. YOUNG[1], M. PAPAKOSTA[2], M. C. HAVRDA[1];
[1]Mol. and Systems Biol., Geisel Sch. of Med. at Dartmouth, Lebanon, NH; [2]Takeda Develop. Ctr. Americas, Inc, San Diego, CA

Abstract: NLRP3 is part of an inflammatory pathway linked to multiple neuroinflammatory diseases including Alzheimer's disease, Parkinson's disease, and multiple sclerosis. NLRP3 activation results in the autocatalytic cleavage of caspase 1 which in turn cleaves pro-inflammatory targets such as IL-1b and pyroptosis mediators such as Gasdermin D. NLRP3 is an important mediator of microglial activation in aging and disease and has emerged as an attractive drug target. To understand the impact of microglial NLRP3 inflammasome activity and provide a pre-clinical system for the analysis of novel therapeutics we developed a new mouse model by combining the microglia-specific CRE-driver, *Tmem*^{119}, with a hyperactive, CRE-dependent, *NLRP3* allele (*Nlrp3*^{L351P}). We confirmed CRE-mediated induction of *NLRP3* expression in brain tissues. Mice were challenged with LPS and inflammation was assayed in plasma and brain tissues. The expression of multiple cytokines was enhanced in brain tissues obtained from of the *Tmem119/NLRP3*^{L351P} mice but not in the plasma, indicating the fidelity of the microglia-specific model system. Treatment of LPS-treated *Tmem119/NLRP3*^{L351P} mice with the peripheral restricted NLRP3 inhibitor MCC950 further identified inflammatory gene expression that was both NLRP3-dependent and specific to the central nervous system. Ongoing characterization of this and similar model systems will allow for determination of cell-type specific activities of the NLRP3 inflammasome. Such insight into neuroinflammatory mechanisms will improve our understanding of NLRP3 biology, impacting the detection and treatment of the many diseases in which NLRP3 has been identified to play a role while providing a platform for testing novel drugs targeting the NLRP3 inflammasome.

Disclosures: E. Carloni: None. **B.E. Rankin:** None. **A.L. Young:** None. **M. Papakosta:** A. Employment/Salary (full or part-time):; Takeda Development Center Americas. **M.C. Havrda:** F. Consulting Fees (e.g., advisory boards); Takeda Development Center Americas.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01DK115526
T32-DK101357

Title: Diet-induced inflammation and inflammatory priming in the nucleus accumbens of male and female rats

Authors: *J. E. FINNELL[1], C. R. FERRARIO[1,2];
[1]Pharmacol., [2]Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Inflammatory cytokines and chemokines have broad-reaching effects on neuronal function and behavior. For example, cytokines and subsequent signaling through p38 mitogen activated protein kinase can impact the synthesis, release, and reuptake of serotonin, dopamine, and glutamate, which in turn can influence mood, motor function, and motivation. Obesogenic diets can produce persistent inflammation in the brain, but current understanding in this area is limited. For example, consumption of a high fat diet (HFD) increases the concentration of interleukin (IL)-1β and tumor necrosis factor (TNF)-α in hypothalamic regions of male rats. Brain regions that influence food-seeking and feeding behaviors, such as the nucleus accumbens (NAc), are also affected by HFD. However, to date only 3 studies have assessed effects of HFD on inflammation in the NAc. These reports, while informative, were limited in the number of cytokines tested and primarily examined effects in males. Additionally, no studies have evaluated HFD-induced effects on inflammatory priming in the NAc, a process of immune sensitization which results in a greater release of cytokines following an inflammatory challenge. As such, there is still much unknown regarding the inflammatory consequences in the NAc following HFD exposure. Therefore, this study aims to broadly characterize the effects of HFD on inflammatory cytokines and neuroinflammatory priming within the NAc of male and female rats. Rats were maintained on either chow or HFD (60%) for 8 consecutive weeks. Food intake and body weight were monitored throughout. NAc tissue was then collected from rats 4 hours following an acute lipopolysaccharide (LPS; 250 ug/kg, i.p.) or vehicle injection (1mL/kg saline, i.p.; N=80; n=10/group). NAc tissue was probed using a panel for 23 cytokines including IL-1β and TNF-α (Bio-Plex multi-plex assay). Beyond changes in the concentration of individual cytokines, diet-induced shifts in cytokine networks was assessed using ARACNE mutual information analysis in conjunction with Cytoscape. Additionally, NAc tissue was probed for high mobility group box-1 and ionized calcium-binding adapter molecule 1 to provide clues about potential mechanisms underlying inflammatory priming and release. Together, these data will provide insights into the effects of HFD on a broad range of inflammatory cytokines within the NAc, mechanisms underlying diet-induced inflammatory priming, and determine whether effects of HFD differ as a factor of sex. These studies are supported by R01DK115526 to CRF and T32-DK101357 to JEF.

Disclosures: J.E. Finnell: None. **C.R. Ferrario:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.25

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI Grant JP19K16623

Title: Behavioral disorders and expression changes of the tyrosine hydroxylase in Cuprizone-induced neuroinflammation mouse model with schizophrenia-like symptoms

Authors: *K. TSUKUDA[1], T. KUBOTA[2], A. CHIBA[3], T. TOMINAGA[1], Y. KISHIMOTO[4], K. NAKASHIMA[1];
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Abstract: A long-term feeding diet containing Cuprizone (CPZ), induces demyelination in the brain (mainly corpus callosum and cingulum of white matter). CPZ, a copper chelator, has been employed to build a mouse model for demyelinating diseases such as multiple sclerosis. On the other hand, it has recently been reported that short-term CPZ treatment induces neuroinflammation without demyelination and psychobehavioral disorders like symptoms of schizophrenia. These facts suggest that short-term CPZ treatment mice may serve as a new animal model for the study of psychiatric disorders with neuroinflammation. However, there have been few reports on the behavioral characteristics of this mouse. In this study, we evaluated the behavioral characteristics of this mouse by spontaneous behavior, social behavior, and prepulse inhibition (PPI), which is a measure of sensorimotor gating and is known to be reduced in schizophrenic patients in addition to histological analyses. These results showed neuroinflammation without demyelination and significant impairment of PPI in short-term CPZ-treated mice as compared with control mice. These results suggest that neuroinflammation induced by short-term CPZ treatment may participate in psychiatric symptoms observed in schizophrenia, such as impaired PPI. To date, the hypo- and hyperactivity of the dopaminergic system are mixed depending on the brain region, and negative and positive symptoms have known that exist simultaneously in schizophrenia. In this study, western blot analyses in the prefrontal cortex, hippocampus, and the midbrain showed that tyrosine hydroxylase was decreased in the prefrontal cortex and increased in the midbrain in inverse proportion to the prefrontal cortex of short-term CPZ-treated mice as compared with control mice. These results suggest that the increase in tyrosine hydroxylase in the midbrain may be a compensatory response to the decrease in dopamine neurotransmission in the prefrontal cortex. Elucidation of the molecular mechanism of PPI dysfunction in short-term CPZ-treated mice may provide a valuable animal model for the study of the pathogenesis mechanism and development of therapeutic agents for schizophrenia.

Disclosures: K. Tsukuda: None. **T. Kubota:** None. **A. Chiba:** None. **T. Tominaga:** None. **Y. Kishimoto:** None. **K. Nakashima:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.26

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sex differences in the protective mechanism of action of caffeine on microglia in a rodent model of preterm hypoxic ischemic injury

Authors: *R. M. MCLEOD[1], R. KOSKI[3], T. ROSENKRANTZ[4], R. FITCH[2];
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Abstract: Though only 11% of infants are born prematurely, a higher proportion of those infants will experience some sort of hypoxic ischemic (HI) injury, a lack of blood and oxygen supply to the brain, which can lead to deficits in motor and cognitive ability. Caffeine is used to treat apnea of prematurity but has also shown to improve cognitive outcomes in premature infants. Though the protective effects of caffeine have been generally established, it is not well known *how* caffeine acts in the brain to be protective. Several studies have indicated that the A1 and A2A receptors are likely targets for protection, but it is unclear if this action is at the neuron or through reducing inflammation. In addition, research has not fully examined for which infants caffeine is best used. This is an important factor as we know there are many things that play into the progression of HI Injuries. We know that female preemies have better outcomes than males, and we also know that hypothermia is a more effective treatment for females (Wood et al., 2020). It is possible that we may see similar sex effects in caffeine treatment. Our study investigates one potential mechanism of protection by caffeine, the A2A receptor on microglia. We examined both female and male rat models of a 32-week gestational age infant given an HI injury using the Rice-Vannucci method. Rodents were treated with caffeine or saline and perfused 48 hours post injury. Their brains were sliced and stained for chromatin condensation, an indicator of cell death, using 4',6-diamidino-2-phenylindole (DAPI), and for microglia using ionized calcium binding adaptor molecule 1 antibody (IBA-1, which binds specifically to microglia). Results indicate that there is less indication of cell death in caffeine treated males and females compared to rodents treated with saline. However, females do not show reduced microglia activation, which is seen in the males of this study. This indicates that there may be differences in the protective mechanism of caffeine for each sex.

Disclosures: R.M. McLeod: None. **R. Koski:** None. **T. Rosenkrantz:** None. **R. Fitch:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.27

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ETSU Quillen Research Enhancement Award

Title: Inhibition of focal adhesion kinase alleviates experimental autoimmune encephalomyelitis

Authors: *C. JIA, C. LOVINS, M. KEASEY, T. HAGG;
East Tennessee State Univ., Johnson City, TN

Abstract: Protecting myeline and axons and promoting remyelination remain crucial therapeutic challenges in multiple sclerosis (MS). Upregulation of extracellular matrix molecules (ECM) and integrin signaling exacerbates inflammatory responses and inhibits remyelination in MS lesions. Blocking integrin binding might be difficult due to ECM abundance and integrin diversity. Pharmacological inhibition of signaling might be better. Focal adhesion kinase (FAK) is a major intracellular mediator of integrin signaling. In a mouse experimental autoimmune encephalomyelitis (EAE) model, we found that EAE activated FAK in the spinal cord and that systemic treatment with a small-molecule FAK inhibitor, FAK14, mitigated clinical signs and reduced demyelination and axonal injury in the early disease phase. FAK14 did not affect T cell infiltration or activation of macrophages and microglia in the brain or change the immune response in the lymph nodes, suggesting that the beneficial effects are mediated by inhibiting FAK in the central nervous system (CNS). Astrocytes are major glial cells in the CNS and affect myelin maintenance and remodeling. Inducible cre-lox knockout of FAK in astrocytes delayed the clinical onset of EAE symptoms and substantially reduced the peak disease severity and chronic disease activity. Importantly, systemic FAK14 treatment during the chronic EAE phase reduced clinical symptoms in wildtype control but not astrocytic FAK knockout mice, suggesting that FAK in astrocytes exacerbates EAE lesions and that FAK14 acts by inhibiting astrocyte FAK. Leukemia inhibitory factor (LIF) and oncostatin M (OSM) are upregulated in the CNS of MS and rodent EAE models and protect myelin and axons. Systemic FAK14 treatment in early EAE upregulated LIF and OSM without altering the expression of the related ciliary neurotrophic factor in the spinal cord. FAK14 did not change LIF and OSM in peripheral immune cells. These data suggest that the beneficial effects of FAK inhibition on EAE might be through upregulation of LIF and OSM in the CNS. Together, this study reveals that inhibition of FAK improves neurological and histological outcomes during the acute and chronic phases of MS and points to opportunities for the rational development of FAK inhibitors, such as FAK14, as a novel MS therapy.

Disclosures: C. Jia: None. **C. Lovins:** None. **M. Keasey:** None. **T. Hagg:** None.

Poster

624. Neuroinflammation: MS, Autoimmune, Other Models

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 624.28

Title: WITHDRAWN

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.01

Topic: C.09.Stroke

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
JSPS KAKENHI Grant (Fostering Joint International Research:18KK0276)
Uehara Memorial Foundation Research Fellowship
Wesco Scientific Promotion Foundation Research Fellowship
Okayama Medical Foundation Research Fellowship

Title: Parvalbumin interneuron circuits mediate functional recovery in post-stroke rehabilitation

Authors: *N. OKABE, T. CARMICHAEL;
635 CHARLES E YOUNG DR S, UCLA NEUROLOGY, LOS ANGELES, CA

Abstract: The damaged brain modifies its neural network to restore function in response to environmental demands. Rehabilitation in clinical practice employs the modification of neural networks. However, it is unclear which neural circuits in the brain are associated with functional recovery.This study aimed to identify neuronal circuits responsible for the experience-dependent recovery after stroke. We investigated synaptic alterations in the rostral forelimb area (RFA) after photothrombotic stroke in the caudal forelimb area (CFA) in the mouse motor cortex using multiple virus labeling approaches (dendritic spine analysis, rabies virus tracing, and GRASP assay). The mice received intensive skilled reaching training (>800 reaches/day, 5days/week, 3weeks) from ten days after stroke. To investigate the involvement of modified neuronal circuits in functional recovery, we also performed acute and chronic chemogenetic studies combined with behavioral assessment by single seed reaching test and grid walk test.Our histological studies revealed that the CFA stroke dramatically reduced dendritic spine density in the neurons projecting to the stroke site (stroke-projecting neurons) from the RFA. The stroke-projecting neurons lost synaptic connections from many brain areas apart from the stroke site, such as the somatosensory cortex, thalamus, and contralesional RFA. These alterations did not occur in the corticospinal neurons in the RFA. Rehabilitative training restored dendritic spine density and part of the synaptic connections. Furthermore, using the rabies virus tracing and the GRASP assay, we identified that the rehabilitative training increased local synaptic inputs from the parvalbumin interneurons (PV-INs) to the stroke-projecting neurons.The acute chemogenetic studies revealed that inhibition of the stroke-projecting neurons disturbed motor function after rehabilitation, suggesting the involvement of stroke-projecting neurons in functional recovery. Furthermore, chronic inhibition of either stroke-projecting neurons or PV-INs blocked functional recovery during the rehabilitative training. These data suggest that neuronal activities in the stroke-projecting neurons and the PV-IN are necessary for functional recovery. Our results suggest that the neuronal circuits formed by the stroke-projecting neurons and PV-INs may play a critical role in the experience-dependent functional recovery after stroke.

Disclosures: **N. Okabe:** 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.; BrainQ, Calico. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BrainQ. **T. Carmichael:** 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.; BrainQ, Calico, Athersys, Fibrobiologics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BrainQ.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.02

Topic: C.09.Stroke

Support: The Lundquist Institute Seed Grant #32530-01

Title: Pre-vascularization of micro-scale hydrogel particles for brain repair after stroke

Authors: *M. MAPUA[1], I. PHAM[1], A. TAHAYERI[2], J. TENG[1], H. PEREZ[1], S. FARHANGNIA[1], L. E. BERTASSONI[2], L. R. NIH[1];
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Abstract: Stroke is the leading cause of disability in the US. The clinical and economical burden of this disease urges the need for a medical solution outside the confines of conventional practices in neurology. While the overwhelming majority of research on brain repair is centered on neurons, an increasing body of evidence suggests that post-stroke angiogenesis plays a fundamental role in guiding repair and functional recovery through the activation of endogenous repair mechanisms. Recent advances in tissue engineering have led to the development of hydrogel materials that can be injected directly into the stroke lesion site to form cell-instructive scaffolds for tissue repair. While these materials offer a potential platform for stem cell transplantation, they have not been actively designed to promote vascular formation in the lesion site or offer protection from the brain's immune response to stroke. To overcome the hurdles of lacking vascularization and acute inflammation, we have recently developed a microfluidic-generated engineered system to transplant vascular cells in the stroke site while reducing the inflammatory response to stroke. This system is a pre-vascularized microporous injectable material made of micro-scale hydrogel building blocks. We found that the when transplanted directly in the lesion site, the cell-free building blocks' immunomodulating effect and ability to promote cellular infiltration are far superior to their bulk nanoporous hydrogel counterpart. In order to prepare the vascular cell-loaded material, we have performed *in vitro* studies in order to identify the optimal cell configuration and clustering presentation of endothelial progenitor cells

(EPC) for vascular formation in a 3D sprouting assay. We generated six distinct cell configurations (n=5/group) in which EPCs are co-cultured with human mesenchymal stem cell (MSCs, EPC:MSC at 1:4 ratio) for 6 days in a fibrin scaffold (3mg/mL fibrinogen, 0.64U/mL thrombin, 10 clusters/gel, 100uL gel). We found that all hydrogels in which EPC and MSCs were cultured within the same cluster (2000 cells/cluster) were associated with a higher number of vascular sprouts (16.14 ± 3.53) compared with conditions in which MSCs were seeded homogeneously in the fibrin gel (12 ± 3.23, $P < 0.005$) or on top of the fibrin gel (11.71 ± 5.05 $P < 0.005$). These results suggest that co-culturing EPCs and MSCs in a clustered conformation at a 1:4 ratio before encapsulation within our HA microgel particles could lead to the highest degree of vascular sprouting and vessel formation in the lesion site brain *in vivo* to promote repair after stroke.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.03

Topic: C.09.Stroke

Support: NIH Grant RFAG042189
NIH Fellowship 1F31NS118970-01A

Title: The Aging Ovary Impairs Acute Stroke Outcomes

Authors: *T. BRANYAN[1], K. F. KOSEL[2], E. LEPE[2], F. SOHRABJI[3];
[1]Texas A&M Univ., Bryan, TX; [2]Neurosci. & Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX; [3]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX

Abstract: Adult female rats (5-7 mos) typically sustain much smaller infarcts after middle cerebral artery occlusion (MCAo) compared to middle-aged females (10-12 mos). This may be due to loss of estrogen, though hormone replacement therapy was shown to increase stroke-related mortality in the Women's Health Initiative. Preclinically, it has been shown that ovariectomizing adult female rats increases infarct volume, and estrogen treatment rescues this effect. Interestingly, ovariectomizing middle-aged female rats and treating with estrogen worsens infarct volume and stroke-induced sensory motor deficits. This study aims to assess what role the aging ovary plays in regulating acute stroke outcome. Reproductively senescent (RS) were subjected to ovariectomy or sham ovariectomy surgery and then allowed to recover for 3 weeks. Animals were then subjected to endothelin-1 (ET-1) surgery to induce a stroke. Sensory motor function was monitored at 2- and 5-days post-stroke using the adhesive removal task (ART) and the vibrissae-evoked forelimb placement task. Animals were terminated at 5d post-stroke, brains

were excised and stained with TTC, and infarct volume was calculated and normalized to the volume of the contralateral hemisphere. Ovaries were also removed and flash frozen, then later processed for protein extraction and analyzed with a Milliplex 27-analyte cytokine panel. OVX animals showed reduced latency in ART at both 2- and 5-days post-stroke, indicating improvement in sensory motor function, compared to intact females. Both OVX and intact animals showed impairment in the VIB task at 2-days post-stroke; however, the OVX group recovered by 5 days. Finally, there was a statistical trend towards a reduction in infarct volume in the OVX group ($p=0.0862$). Moreover, analysis of 27 cytokines showed that five inflammatory cytokines (IL-1β, IL12p70, IFNγ, IL-18, and RANTES) were decreased in the aging ovary in the animals that underwent ET-1 surgery compared to shams. Such a decrease was not observed in young ovarian protein. These results indicate that secretions of the aging ovary impair stroke recovery, and that the aging ovary responds to stroke either directly or indirectly via extravasation of immune cells into circulation. These data suggest the novel hypothesis that stroke severity in aging females may be mediated by the aging ovary.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

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Program #/Poster #: 625.04

Topic: C.09.Stroke

Support: NIH grant R41 NS056626
NIH grant R42 NS112070
NIH training grant T32 DA007097
Suzanne Schwarz Fund

Title: Translation of Human Umbilical Cord Blood Derived Stem Cells to the Clinic for Treating Stroke

Authors: *S. R. VAR[1], M. SHIAO[1], V. KRISHNA[2], G. BADGER[1], N. EMMITT[2], C. DAY HAM[1], D. CHEN[1], A. CRANE[3], M. CHEERAN[2], N. KUZMIN-NICHOLS[4], A. GRANDE[1], W. LOW[1];
[1]Neurosurg., [2]Vet. Population Med., [3]Pediatrics, Univ. of Minnesota, MINNEAPOLIS, MN; [4]Saneron CCEL, Tampa Bay, FL

Abstract: Human umbilical cord blood (hUCB) is a rich source of stem cells that has been evaluated for the treatment of various disorders and diseases. We have isolated a CD34-negative population of stem cells from hUCB that exhibits neuroprotective properties in experimental animal models of ischemic brain injury. We conducted IND-enabling studies in consultation with panel members at the U.S. Food and Drug Administration in order to translate our non-hematopoietic umbilical cord blood stem cells (nhUCBSCs) to the clinical setting. We carried

out toxicity, tumorigenicity, and therapeutic efficacy studies on nhUCBSCs to meet the milestones for FDA approval for a Phase I Clinical Trial. Immunocompetent Wistar rats were injected intravenously with $1x10^6$ nhUCBSCs. For the toxicity studies, adverse events such as seizures, respiratory distress, decreased weight, and death were monitored. Serum cytokines were monitored to determine if the administration of the cells led to systemic inflammation. For the tumorigenicity studies, karyotyping of the nhUCBSCs were conducted at different passages to assess chromosomal translocation, and telomerase activity was evaluated in nhUCBSCs. The presence of possible tumors in the various organs throughout the body of each animal was evaluated. The biodistribution of nhUCBSCs after intravenous administration was assessed. For the therapeutic efficacy studies, animals were subjected to a middle cerebral artery occlusion (MCAO) and administered nhUCBSC 2 days following the ischemic brain injury. Animals treated with nhUCBSCs and vehicle controls were tested on a battery of behavioral tests after MCAO, which included the modified neurological severity score (mNSS) and performances on the vertical pole. Our findings showed that there were no signs of toxicity after the administration of nhUCBSCs, including no adverse events observed and no secretion of inflammatory cytokines. Our biodistribution results showed that nhUCBSCs migrated to various organ systems at early time periods, localizing in the bone marrow and spleen in 2 hours, then disappeared by 48 hours. There was no evidence of tumorgenicity, in which no tumors were observed in any organ system, nor was there chromosomal translocation in nhUCBSCs. Our therapeutic efficacy studies showed that treatment with nhUCBSCs 2 days after MCAO significantly improved behavioral outcomes in both modified neurological severity scores and vertical pole tests 28 days after the ischemic event.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.05

Topic: C.09.Stroke

Support: The Lundquist Institute Seed Grant #32530-01

Title: Engineered pro-angiogenic hydrogel for neural progenitor cell transplantation after stroke

Authors: *I. PHAM[1], M. MAPUA[1], J. TENG[1], M. NAVARRO[1], H. PEREZ[1], L. R. NIH[1,2]; [1]The Lundquist Inst., Torrance, CA; [2]Neurol., UCLA, Los Angeles, CA

Abstract: Stroke is the leading cause of disability in the US. To date, no clinical trials have succeeded in alleviating stroke survivors' neurological impairment. Stem cell transplantation after stroke has shown promise in promoting the activation of brain repair mechanisms in pre-

clinical models of cerebral ischemia but has not advanced to a clinically relevant treatment due to poor cell survival rates and lack of control over cell differentiation. We have recently developed an injectable extracellular matrix (ECM)-mimetic hydrogel platform for neural progenitor cell (NPC) transplantation in the stroke brain using a DOE (Design of Experiment)-based systematic approach to optimize the material's capacity to control stem cell survival and differentiation into specific neural lineages *in vivo*. These findings successfully led to a unique hyaluronic acid (HA) hydrogel formulation specifically designed to promote the highest degree of cell survival (HA Max) both *in vitro* and *in vivo*. Although this technology significantly improved cell viability, it was not associated with enhanced functional recovery due to a lack of vascularization of the transplanted graft. To address this limitation, we have developed a nanotechnology in which Vascular Endothelial Growth Factor (VEGF) is clustered and immobilized onto heparin nanoparticle's surface. We found that Highly Clustered VEGF (hcV) promotes vascular sprouting *in vitro* and *in vivo*. When loaded into an HA hydrogel and injected within the infarct core of a mouse model of stroke, hcV stimulates the formation of mature and functional vascular and axonal networks. In this study, we merge our hcV technology with our pro-neuronal hydrogel formulation to enhance the pro-angiogenic capability of the NPC graft and promote NPCs long-term survival and integration in the host tissue. To evaluate the direct effect of hcV on NPCs, we cultured NPCs *in vitro* (n=5/group) within HA Max with and without soluble VEGF (Vs) or hcV (200 ng/ 5 uL gel) for 6 days. Our results show that both HA Max + Vs and HA Max + hcV conditions were associated with a significant increase in cell viability compared to HA Max alone (79.05% ± 13.15 vs. 85.48% ± 7.47, respectively, $P<0.01$), as well as cell proliferation (285% ± 47.25, $P<0.001$ vs. 88.87% ± 29.68, $P<0.05$ respectively). In addition, HA Max + hcV showed a significant decrease in Nestin positive cells in comparison to HA Max + Vs (47.47% ± 6.91 vs. 77.95% ± 13.49, respectively, $P<0.001$), suggesting that hcV stimulates NPCs' quiescence exit to guide their differentiation. Our results suggest that the addition of hcV to HA Max might enhance long-term survival and tissue integration of the transplanted graft *in vivo*.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.06

Topic: C.09.Stroke

Support: Internal ASU grants

Title: Word repetition paired with exposure to startling acoustic stimuli improves aphasia and apraxia of speech in individuals with severe-to-moderate stroke: a stratified, single-blind, randomized control trial.

Authors: *Z. E. SWANN[1], C. F. HONEYCUTT[2];
[1]Neurosci., Arizona State Univ. - Tempe Campus: Arizona State Univ., Phoenix, AZ; [2]Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

Abstract: OBJECTIVES: The objective of this study is to evaluate START (Startle Adjuvant Rehabilitation Therapy) as a non-invasive and cost-effective tool for treating individuals with severe post-stroke speech impairment. Post-stroke speech is susceptible to a phenomenon known as StartReact, by which prepared movement is elicited via a startling, acoustic stimulus (SAS). Training with StartReact enhances post-stroke reach performance. Here, we evaluate if training with StartReact would enhance speech planning and execution in individuals with post-stroke speech impairments. METHOD: Individuals with post-stroke aphasia, apraxia, and dysarthria were trained with (START; N=21) or without (CONTROL; N=21) a SAS in a blinded randomized control trial. Training consisted of 3 days of remotely-delivered high-frequency auditory-repetition of words. Subjects were instructed to GET READY to say a target word and to GO. The START group received a 105 dB white noise SAS in place of a GO cue one-third of the time. Clinical measures (WAB-R, ABA-2, SIS) were evaluated before (Day 1) and after (Day 5) training. RESULTS: Apraxia severity was the best predictor of START efficacy. Those with moderate to severe apraxia receiving START had improved Comprehension (p=0.006), Repetition (p=0.05), Word finding (p=0.035), Reading (p=0.020), Diadochokinetic rate (p=0.003), Word accuracy (p=0.004), and Picture naming speed (p=0.027). The START group reported higher perceived recovery in communication (SIS) (+15.2%, p=0.01) compared to Controls who saw no change in any subtest. Mild subjects showed no change in both test and control groups. The overall aphasia quotient did not improve by a clinically meaningful difference (+5 points) in any group. CONCLUSIONS: We are the first to evaluate START as a therapy for post-stroke speech. While prior StartReact studies saw execution gains during SAS in a variety of disorders and tasks, we show, for the first time, changes to language and speech motor planning after only 3, 90-minute sessions of SAS exposure. The results of this clinical trial indicate that START can be deployed remotely and may prove a valuable, adjuvant tool to improve functional speech for individuals with severe impairments who might not be eligible for traditional speech therapies.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.07

Topic: C.09.Stroke

Support: Korean Disease Control and Prevention Agency (2022-11-006)
National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-2020R1A2C3010304).

Title: Long-term Recovery Patterns of the Multi-faceted Functional Domains in the Stroke Survivors: The KOSCO Study

Authors: *S. SHIN[1], Y. LEE[1], W. CHANG[1], M. SOHN[2], J. LEE[2], D. KIM[2], Y.-I. SHIN[2], G.-J. OH[2], Y.-S. LEE[2], M. JOO[2], S. LEE[2], M.-K. SONG[2], J. HAN[2], J. AHN[2], S. CHOI[3], S. LEE[3], Y.-H. KIM[1,4];
[1]Samsung Med. Ctr., Samsung Med. Ctr., Seoul, Korea, Republic of; [2]The Korea Stroke Cohort for Functioning and Rehabil. Res. Group, Seoul, Korea, Republic of; [3]Korea Dis. Control and Prevention Agency, Cheongju, Korea, Republic of; [4]SAIHST, Sungkyunkwan Univ., Seoul, Korea, Republic of

Abstract: Stroke produces long-term residual disabilities for diverse aspects of human functions, such as movement, cognition, language, and swallowing. The recovery or late declining patterns differ by each functional domain. The purpose of this study was to determine the long-term functional recovery patterns in first-ever stroke survivors. This study is an interim analysis of the Korean Stroke Cohort for Functioning and Rehabilitation designed for follow-up of first-ever stroke patients. Out of 10,636 patients screened, 7,858 patients agreed for long-term follow up. Among them, 4,443 patients completed face-to-face functional assessments 10 times from 7 days to 5 years after stroke onset and included for this analysis. We analyzed recovery patterns of six functional domains such as motor, ambulatory, cognition, language, swallowing, and activities of daily living functions for 5 years since stroke onset. Generalized estimating equations (GEE) analysis was done for subgroups according to age, stroke type, and initial stroke severity, respectively. Among all patients, 3,508 patients (78.96%) suffered from ischemic stroke (IS) and 935 patients (21.04%) hemorrhagic stroke (HS). Overall, functional levels reached to plateau at 12 or 18 months after stroke onset and then slightly declined after 30 months after onset. The GEE analysis showed significant difference in functional recovery pattern between subgroups. Patients with severe stroke or with HS showed stiffer early recovery slopes during the first 3 months than mild to moderate stroke or IS, respectively. Patients older than 65 years showed significant decline in all functional domains after post-stroke 36 months. Understanding different functional recovery patterns according to functional domains, age, stroke type, and initial stroke severity will help clinicians establishing optimal stroke care strategies.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.08

Topic: C.09.Stroke

Title: Identifying alterations in muscle synergies and motor unit recruitment in stroke survivors undergoing task-oriented therapy

Authors: N. TACCA[1], B. R. SCHLINK[1], M. J. DARROW[1], **E. C. MEYERS**[1], D. A. FRIEDENBERG[2], *D. J. GABRIELI[2];
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Abstract: In the United States, approximately 5 million people are living with hemiparesis that limits their functional independence and creates reliance on caregivers for essential activities of daily living. Restoration of hand and wrist function after stroke is a critical unmet need, as only 5-20% of stroke survivors with hemiparesis regain complete function in their affected arm despite months of conventional rehabilitation therapy. As novel methods emerge to enhance the current state of rehabilitative care, including advancements in functional electrical stimulation, robotic prosthetics, and neuromodulatory techniques, methods to track recovery in these individuals becomes of critical importance to monitor recovery and guide therapy.
Non-invasive electromyography (EMG) biomarkers have recently showed promise in understanding the changes that occur in neuro-muscular control after stroke and throughout therapy. Muscle synergies, the patterns of muscle co-activation that produce specific movements, have been shown to be altered in the paretic forearm following a stroke. Moreover, the profiles of muscle synergies in the paretic arm trend toward those in the non-paretic arm as stroke patients regain functional mobility through rehabilitation, which suggests that muscle synergies may serve as a useful biomarker for stroke recovery. Additionally, the neural drive to the paretic side of the body is reduced following a stroke and results in decreased motor unit (MU), the fundamental component of neuro-muscular activation, recruitment. Specifically, the number of detectable MUs, average MU firing rate, MU firing coherence are altered in the paretic arm after stroke.
Using a 150-electrode high-density surface EMG garment, we highlight differences in the forearm muscle synergies and MU recruitment between able-bodied individuals (N=7), chronic (N=3), and sub-acute stroke survivors (N=3) undergoing intensive rehabilitation protocols. We show the movement specific spatiotemporal dynamics of muscle synergy patterns and activation profiles are altered in individuals after stroke and track with the course of therapy. Similarly, MU activations show differences in both temporal and frequency domains that relate to clinical measures of functional impairment and stroke phase. Together, these results suggest that muscle synergies and MU activity derived from surface EMG can be sensitive measures of the functional impairment after stroke and may be used to monitor and guide therapy.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.09

Topic: C.09.Stroke

Support: Tobacco-Related Diseases Research Program (TRDRP), University of California, Office of the President (UCOP) # T32IR5390

Title: Effect of perinatal nicotine exposure on the development of the cerebrovascular network in the offspring

Authors: *A. K. PENA, I. PHAM, M. MAPUA, H. PEREZ, V. K. REHAN, L. R. NIH;
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Abstract: According to the Centers for Disease Control and Prevention (CDC), 20.8% of the US adult population smokes. While cigarette smoking doubles the risk of cerebral ischemia, it is also associated with a unique response to stroke as heavy smokers present larger brain lesions, aggravated neurological deficit, and delayed recovery compared with non-smoking patients, suggesting that cigarette smoking impairs the activation of the brain's endogenous repair mechanisms typically associated with recovery. An increasing body of evidence links angiogenesis to repair and functional recovery after stroke. Therefore, identifying the mechanisms that hinder vessel formation in the injured brain is a critical step to developing therapeutic approaches that efficiently improve recovery after stroke. According to the National Center for Health Statistics, one in 14 women who gives birth in the United States smokes cigarettes during pregnancy. Although maternal smoking has been linked to a variety of negative infant and child outcomes, its impact on the cerebrovascular development in the offspring remain unknown. In this study, we propose to explore the impact of nicotine, the principal constituent of both traditional and e-cigarettes, on the formation and remodeling of the brain vascular network in exposed-offspring. Our *in vivo* preliminary data show that nicotine-exposed offspring exhibit a cerebrovascular impairment with decreased vascular density (16.21 ± 3.93 vs 28.13 ± 2.84 in non-exposed offspring, $P<0.005$) and a morphologically impaired vascular network. These findings are associated with a decreased number of subventricular neural progenitors (14.33 ± 2.45 vs 21.78 ± 1.06 in non-exposed offspring, $P<0.005$). In addition, we found that concomitant treatment of nicotine with the peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist rosiglitazone (RGZ) normalized both angiogenesis (24.59 ± 3.29) and neurogenesis (17.69 ± 0.96) in exposed brain. These results show that perinatal exposure to nicotine can alter the development of the cerebral vasculature and affect neuronal growth in the developing brain, and suggest that exposed offspring could exhibit an impaired brain repair machinery, leading to delayed healing and recovery after stroke. In addition, our findings identify RGZ as a potential therapeutic target to prevent nicotine-mediated effects on nicotine-exposed offspring.

Disclosures: **A.K. Pena:** None. **I. Pham:** None. **M. Mapua:** None. **H. Perez:** None. **V.K. Rehan:** None. **L.R. Nih:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.10

Topic: C.09.Stroke

Support: AHA 831331

Title: Intestinal Epithelial Stem Cell Transplants as a Novel Therapy for Cerebrovascular Stroke

Authors: *K. MANI[1], Y. EL-HAKIM[2], T. BRANYAN[3], N. SAMIYA[4], F. SOHRABJI[5]; [1]Texas A & M Univ., Bryan, TX; [2]Texas A&M Hlth. Sci. Ctr., Bryan, TX; [3]Texas A&M Univ., Bryan, TX; [4]Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., College Station, TX; [5]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX

Abstract: Stroke is a leading cause of disability and dementia with limited treatment options. Evidence suggests that stroke rapidly dysregulates the intestinal epithelium, causing a 'leaky gut' and elevated blood levels of inflammatory cytokines. Here we tested whether transplantation of intestinal epithelial stem cell (IESC), which repair the gut, would also improve stroke recovery. Middle-aged female Sprague-Dawley rats were assigned to the following groups: Sham (no stroke or treatment), stroke with vehicle transplant; stroke with IESC transplants. Rats were subjected to middle cerebral artery occlusion (MCAo) by stereotaxic guided injection of Endothelin-1. Primary IECs were isolated from young female rats to prepare organoids cultures. Dispersed organoid cells were injected iv 4h/24h/48h after stroke. In the acute phase (4d), sensorimotor function was assessed by the adhesive tape removal test. In the chronic phase (4 weeks after stroke) animals were assessed for depressive-like behaviors (Social Interaction, burrowing test) and cognitive function (Novel Object Recognition Task, and Barnes maze test). All behavior analysis were scored by experimenters who were blind to the treatment condition. Serum levels of the LPS and IL-17A were quantified by ELISA. MCAo impaired ART performance at 2d and 4d after stroke in the vehicle treated group, but no impairment was noted in the group that received IESC transplants. At 4 weeks after stroke, vehicle-treated stroke animals showed significantly decreased displacement of wood chips in the burrowing test as well as decreased time time spent with a conspecific in the social interaction test, indicating depressive-like behaviors. IESC-transplanted animals showed no change in these behaviors compared to their pre-stroke levels. A similar pattern was noted in the NORT test where vehicle-treated animals failed to discriminate the novel object after stroke . In the Barnes maze, learning and recall of the escape hole was also impaired in this group, indicating that focal ischemia impaired cognitive function. IESC-transplanted animals displayed no impairment in these cognitive tasks. IESC treatment also reduced levels of serum LPS and IL-17A in the acute and chronic stage of stroke, which were elevated in the vehicle group, suggesting a potential mechanism for IESC therapy. These data show that early intervention can alleviate stroke-induced cognitive/affective impairment, and demonstrate the effectiveness of gut stem cell therapy.

Disclosures: K. Mani: None. **Y. El-Hakim:** None. **T. Branyan:** None. **N. samiya:** None. **F. Sohrabji:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.11

Topic: C.09.Stroke

Title: Enhancing use of queued speech for functional treatment of pure expressive aphasia

Authors: *E. L. ALTSCHULER[1], I. Y. BALKAYA[2];
[1]PM&R, Metropolitan Hosp. Ctr., New York, NY; [2]PM&R, Metropolitan Hosp., NY, NY

Abstract: Aphasia is a common and severely debilitating consequences of stroke and other brain disorders. Currently there are no good treatment options available for aphasia. Traditionally aphasia has been divided into two main types, expressive (Broca's) and receptive (Wernicke's) aphasia. Patients with expressive aphasia have difficulty predominantly with propositional speech—spontaneous speech that uses vocabular for a specific purpose—secondary to lesion in left frontal hemisphere, They also have paucity speech. Patients with receptive aphasia can produce fluent speech but typically it is nonsense gibberish with paraphasias and other errors. Recently, the importance of a third deficit, actually of speech not language, has been appreciated, apraxia of speech (AoS)—difficulty coordinating muscles to produce speech. This was first described in 1969 by Darley. Patients with pure expressive aphasia have full understanding and are able to speak with normal articulation when repeating phrases as Wernicke's area and area 55b in the left frontal lobe (the AoS area) are intact. However, these patients have severe difficulty producing propositional speech. Recently, we noted (Balkaya et al., & Altschuler, *Ann Indian Acad Neurol.* 2021) that in addition to intact repetition, patients with pure expressive aphasia can sequence i.e., given "1,2,3," they can keep counting up to 10 or further, list days of the week and months of the year. They can "super sequence," i.e., given "2,4" they can continue up to 10 and beyond. We have seen a patient with pure expressive aphasia who can do all these tasks and also read with perfect comprehension. Propositional speech was a significant problem—when asked what she had for breakfast, even looking at foods she said, "I had," but could speak no further. We tested the patient with food ordering task, over the phone using a menu from a local diner. When asked what she would like for lunch, she picked out and read to us "a burger and toppings." Extemporaneously, we asked her how she wanted her burger to cooked. She responded "extremely rare!" This was listed nowhere on the menu. We believe her response was not true proposition speech, but was somehow queued by the question, based on the patient's long experience being asked this. This specific example may point to a generally useful method to enhance functional speech—utilize queued speech that can be performed by Wernicke's area without assistance from Broca's area. A general training method, borrowing from reinforcement learning theory of artificial intelligence, would consist of engaging a patient in conservation, watching TV, listening to others, and reading to thus build up a stock of queued responses.

Disclosures: E.L. Altschuler: None. **I.Y. Balkaya:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

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Program #/Poster #: 625.12

Topic: C.09.Stroke

Support: NIH BRAIN Initiative Diversity Supplement UH3NS100541

Title: Using HD-EMG to detect individualized stroke-related differences in H-reflex recruitment patterns

Authors: *E. BEDOY[1], E. CARRANZA[2], K. LEITHOLF[1], G. F. WITTENBERG[3], E. PIRONDINI[4], D. WEBER[5];
[2]Bionegineering, [3]Neurol., [4]Rehabil. and Neural Engin. Labs., [1]Univ. of Pittsburgh, Pittsburgh, PA; [5]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Motor impairments are common after stroke and include muscle weakness, abnormal synergies, and spasticity. A variety of rehabilitation strategies are available and should be selected to target specific deficits. However, this is challenging because the location and extent of brain damage tends to vary widely between stroke patients. Optimal outcome relies on specific therapy that is personalized but with each treatment requiring a long time commitment, it is impractical to try all approaches, especially when recovery is the greatest in the first 6 months after stroke. We propose a technique that could potentially provide a rapid, yet detailed measurement of the functional status of sensorimotor neural circuits affected by stroke. When reapplied regularly, throughout a specific therapeutic regime, we can track changes in the neural circuits to determine whether to continue a course of action or try an alternative. Here, we tested for differences in H-reflex recruitment patterns among stroke patients with mild motor impairments. A pair of high-density EMG (HD-EMG) arrays were placed on the extensor and flexor side of the forearm to measure responses evoked by stimulation of the median and radial nerves. The hand opened and closed positions were sustained to preferentially facilitate H-reflexes in relevant muscles, thereby, allowing us to map muscle synergies during two commonly used and opposing hand positions. Our results showed that there were apparent differences in response patterns between the healthy and stroke groups despite all functional assessment scores reaching ceiling values. Three stroke patients had abnormally enhanced flexor H-reflexes, one of which had absent extensor H-reflexes, when the hand was opened. There were also pattern differences across stroke patients (i.e. different flexor muscles were enhanced) highlighting the sensitivity of HD-EMG at identifying individualized corticospinal changes. Our study demonstrated that H-reflex recruitment patterns can be a potentially useful metric for identifying neurophysiological changes after stroke and may serve as a marker to identify the optimal rehabilitation approach for an individual.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.13

Topic: C.09.Stroke

Support: University of Toronto Graduate Student Bursary

Title: Rhythmic music-based intervention promotes chronic stroke recovery via improved paretic limb acceleration irrespective of lesion location

Authors: *T. LORIA[1], C. HAIRE[1], V. VUONG[2], J. DE GROSBOIS[4], L. TREMBLAY[3], M. H. THAUT[5];
[1]Fac. of Music, [3]Fac. of Kinesiology & Physical Educ., [2]Univ. of Toronto, Toronto, ON, Canada; [4]Rotman Res. Inst., Baycrest Hlth. Sci., Toronto, ON, Canada; [5]Fac. of Music and Fac. of Med., Univ. iof Toronto, Toronto, ON, Canada

Abstract: Lesion location can impact motor impairment, which influences the effectiveness of stroke rehabilitation. This exploratory study assessed the effect of a rhythmic music-based intervention (i.e., Therapeutic Instrumental Music Playing - TIMP) on upper-limb motor recovery in different lesion locations and stroke types. Data from 21 chronic stroke patients enrolled in a larger clinical trial were used. CT scans grouped participants according to lesion location: frontal lobe (n = 6), middle cerebral artery (n = 7), pons/brain stem (n = 5), and basal ganglia (n = 3). Data were also grouped by stroke pathology including ischemic (n = 9) and hemorrhagic (n = 12). Participants were assessed at baseline (i.e., pre-test) and post-test using the Fugl-Myer Upper Extremity (FM-UE) and Wolf Motor Function (WMFT) tests. An accelerometer was worn on the paretic limb during training. Accelerometry data was used to compute peak power, a measure reflecting the intensity of paretic limb accelerations. During training, rhythmic auditory cues were provided to retrain paretic arm control using musical instruments as movement endpoints. An interactive sound tablet that provided real-time auditory feedback of paretic limb movements was also used. Data analysis first compared pre- to post-test FM-UE and WMFT scores as well as peak power values between sessions (S) 1 and 9 for the total sample. Wilcoxon signed-rank tests revealed the median scores for all participants improved from pre- to post-tests on the FM-UE (pre-test = 31.5; post-test = 38.5) and WMFT (pre-test = 28; post-test = 32.5). A one-way ANOVA for peak power revealed paretic limb accelerations increased from S1 to S9 (S1 = .04 G^2/Hz; S9 = .2 G^2/Hz) in frequency ranges associated with intentional movement (e.g., 4 Hz). To ascertain whether lesion location and stroke type influenced the magnitude of improvement, a percentage of change score was computed by dividing the post-test scores (i.e., S9 for peak power) by the sum of the post-test minus the pre-test score (i.e., S1 for peak power). Kruskal-Wallis H tests revealed no significant differences in the magnitudes of FM, WMFT, or peak power changes between stroke location groups. Similar nonsignificant results were found for stroke pathology. The results may suggest that despite differing lesion locations, motor recovery was consistent after TIMP. This

consistency may have been driven in part by the provision of rhythmic auditory cues that provided stable spatiotemporal feedback for neural entrainment of paretic limb acceleration. Thus, rhythmic music-based interventions focused on paretic limb acceleration may facilitate neuromotor recovery irrespective of lesion location.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.14

Topic: C.09.Stroke

Support: Lundquist Institute Seed Grant #32530-01
CIRM Research Training Grant / Pre-doctoral Fellowship #EDUC4-12837

Title: Immunomodulating hydrogel material for the brain transplantation of vascular progenitors after stroke

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[2]Neurol., UCLA, Los Angeles, CA

Abstract: Stroke is a leading cause of adult disability and the fifth cause of death in the United States and no clinical trial have succeeded in promoting brain tissue regeneration. Stem cell transplantation has shown promise in enhancing brain repair in pre-clinical stroke models, however the clinical translation of cell therapies is technically challenging on multiple levels: 1) Transplants in the stroke brain are limited by poor survival due to the massive inflammatory response to stroke, 2) Cells are often transplanted in suspension, deprived of adhesive support and growth factors necessary for cell survival and differentiation, 3) Poor vascularization of the graft significantly limits its long-term survival and integration within the host tissue. In order to overcome these limitations, we have developed an injectable nanoporous hyaluronic acid (HA) hydrogel-based engineered system specifically designed to transplant vascular progenitor cells directly within the stroke lesion while releasing Cannabidiol (CBD) to promote immunomodulation. CBD, a cannabinoid with non-psychotropic neuroprotective effects, exhibits anti-inflammatory and antioxidant properties in preclinical models of brain injury and stroke. In order to deliver the molecule in a controlled-release manner, we encapsulated CBD in Pluronic micelles (0.2 µg CBD/µL gel; 30 nm diameter) actively designed to covalently bind with the HA backbone. In our study, we used RGD-functionalized HA-acrylate hydrogels (3.5% HA) crosslinked using a di-thiol MMP9-sensitive crosslinker (65% Acrylate/SH) and a photo-thrombotic mouse model of cortical stroke. We found that once crosslinked, CBD micelle-loaded HA hydrogels retain a modulus of ~400 Pa (476.61 ± 17.69 compared with 443.42 ± 16.13 in

micelle-free HA hydrogels, n=4/group) which mimics the mechanical properties of the mouse cortex. Using *in vivo* fluorescence imaging and encapsulated fluorescent dyes, we found that drugs released from micelles are detectable for a longer time (42% compared with drugs released from HA hydrogels only) and diffuse to a larger area of the brain (44.05 ± 0.08 mm^2 compared with 25.79 ± 0.87 mm^2 in micelle-free hydrogels, n=3/group). In order to encapsulate vascular progenitor cells within the CBD-hydrogel, human umbilical vein endothelial cells (HUVECs, 3000 cells/uL) were mixed with the CBD-micelle-HA solution before crosslinking, and were injected directly within the stroke cavity (5 µL/mouse). Our data show that the presence of CBD-micelles does not affect HUVECs cell survival and vessel formation in both 3D sprouting assay *in vitro* and in the injected brain *in vivo*.

Disclosures: H. Perez: None. **M. Mapua:** None. **I. Pham:** None. **L.R. Nih:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.15

Topic: C.09.Stroke

Support: American Heart Association Predoctoral Fellowship (900190)

Title: Bimanual vs unimanual rehabilitative training: patterns of activity-dependent structural plasticity after stroke

Authors: *V. NEMCHEK, C. J. HOANG, D. SUNDARARAMAN, T. A. JONES;
Univ. of Texas Austin, Austin, TX

Abstract: After motor cortical strokes in rodents, compensation with the less-affected forelimb alters patterns of reorganization in the peri-infarct cortex. These structural changes are associated with worsened functional recovery of the impaired forelimb. Could compensatory use of the less-affected forelimb, and subsequent neuro-structural changes, be advantageous under the right conditions? It has been proposed that coordinated use of both forelimbs may capitalize on the ease of using the less-affected forelimb without jeopardizing functional recovery of the impaired forelimb post-stroke. However, neural underpinnings of both impaired forelimb rehabilitation and coordinated use of both forelimbs remain unclear.
This study determines how bimanual, compared to unimanual, motor rehabilitative training impacts synaptic structural plasticity in the peri-infarct cortex and homotopic contra-lesion cortex after photothrombotic sensorimotor cortical stroke in Thy1-GFP mice. Cranial windows were implanted bilaterally. This novel application allows the use of *in vivo* 2 photon imaging to compare dendritic spine dynamics of cortical pyramidal neurons within individuals across both hemispheres and rehabilitative time. For 4 weeks after photothrombotic cortical infarcts, mice were trained on either their impaired forelimb unimanually or both forelimbs concurrently. The single seed reaching task was used as unimanual rehabilitative training (and assessment of

forelimb function). Bimanual training consisted of another skilled reaching task in which food rewards were retrieved with cooperative use of both forelimbs.
Simultaneous activity-dependent plastic changes in both hemispheres of the brain are expected to underpin to the beneficial effect of bimanual training on impaired forelimb function. As such, functional recovery of the impaired forelimb post-stroke should be positively related to degree of plastic change (as measured by dendritic spine density, turnover, and stability). While previous work has linked increases in contra-lateral plasticity to poorer functional recovery, we expect that mice trained bimanually may be able to utilize such changes to facilitate functional recovery of the impaired forelimb. This study illuminates neuroplastic patterns underpinning different rehabilitative activities and functional outcomes.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.16

Topic: C.09.Stroke

Support: Veterans Administration Merit Award # I01 RX002981

Title: Direction of motion discrimination training after V1+ lesions: fMRI analysis in 3 subjects

Authors: *A. RINA[1,2], A. PAPANIKOLAOU[3], G. A. KELIRIS[4], S. M. SMIRNAKIS[1];
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Abstract: After a stroke or lesion to the primary visual cortex, dense contralateral hemianopia or quadrantanopia develops, in which conscious visual awareness is lost. Visual motion discrimination training has been shown to generate considerable retinotopically specific recovery inside the scotoma. Approximately half of our patients recover in this task after receiving rehabilitative training inside the scotoma; nevertheless, it is unclear why some subjects recover while others do not; also, the mechanism of recovery, when it occurs, is unknown. We aim to study this question in a patient population with V1+ lesions. *Objectives:a*. Determine how strongly motion-responsive visual regions respond to RDK stimulation at a variety of motion coherences. Compare the RDK response profiles before and after training at the trained site, as well as between trained and untrained visual field locations. b. Characterize changes in the visual cortex organization that occur as a result of training using fMRI population receptive field (pRF) mapping methods. Our long-term goal is to describe the recovery mechanism and determine which visual field areas may have greater rehabilitation potential. We present preliminary results

from 3 patients (SP1,SP2,SP3). SP1 had a left PCA stroke and residual right homonymous hemianopia, SP2 had also RHH caused by spontaneous bilateral subdural hematomas and SP3 left superior quadrantanopia due to right occipital stroke. For direction-of-motion discrimination training, we used Random Dot Kinematogram Stimuli (RDK) at different motion coherences, as described in Huxlin *et al.* (2009); two locations inside the dense region of the scotoma were selected, one for training the other for control. Subjects were eye tracked at home using a Tobii 4C portable eye tracker and in the lab using (Eyelink 1000+). fMRI scans were carried out in a SIEMENS (Prisma) 3T scanner with a 20-channel coil under infrared eye tracking (ViewPoint®). For pRF and retinotopy mapping, high-contrast checkerboard bar stimuli were used, while RDK stimuli were used for measuring motion response functions. According to our preliminary data, two patients' formal Humphreys Visual fields improved, while the third reported subjective improvement. We found that visual rehabilitation training in a motion direction discrimination task increased the patients' performance and enhanced responses hV5/MT+.

Disclosures: **A. Rina:** None. **A. Papanikolaou:** None. **G.A. Keliris:** None. **S.M. Smirnakis:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

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Program #/Poster #: 625.17

Topic: C.09.Stroke

Support: National Institute of Health R01 NS103788
California Institute of Regenerative Medicine TRAN1-12891, DISC2-09018 and DISC2-12169

Title: Clinical Translation of Allogenic Regenerative Cell Therapy for White Matter Stroke and Vascular Dementia

Authors: *S. AZARAPETIAN[1], E. HATANAKA[1], J. GARCIA[1], W. E. LOWRY[2], S. T. CARMICHAEL[1], I. L. LLORENTE[1];
[1]Neurol., [2]Molecular, Cell and Developmental Biol., UCLA, Los Angeles, CA

Abstract: Stroke is the leading cause of adult disability. It continues to increase, paralleling the aging population, regardless of the improved acute stroke care to battle the associated debilitation and death rate. White matter stroke (WMS) is due to the occurrence of small infarcts in deep penetrating blood vessels of the brain, affecting brain regions responsible for connectivity. The immediate and imminent consequences of WMS include death of glial cells, gait abnormalities, and challenges in executive functioning, presenting as vascular dementia (VaD). These permanent damages produce an urgent need to explore and treat this neurodegenerative disease. Here, we present a stem cell therapy designed as an allogenic off-the-

shelf product to allow recovery of WMS in affected patients. Immunodeficient mice had a WMS induced via focal microinjection of a vasoconstrictor N5-(1-iminoethyl)-L-ornithine (L-NIO), leading to damage of axons, myelin, astrocytes, and oligodendrocytes. These cellular changes induce motor and cognitive deficits in mice, as seen in humans. Pluripotent stem cells from the fibroblast tissue of four distinct human donors were differentiated into glial enriched progenitor cells (hiPSC-GEPs) and transplanted independently into the stroke core 7 days post-WMS. We ascertained resultant behavioral improvement by tracking shifts in gait and measuring upper limb use tendencies, via grid-walking and cylinder behavioral tasks, respectively. Furthermore, regardless of which hiPSC-GEP donor line the cells were derived from, they all led to motor improvements after stroke. These results, alongside testing with multiple donors and multiple clones from the same donor demonstrate that our cell differentiation process is very robust. The proposed mechanism for this functional improvement is transplant-induced remyelination and axonal regeneration from the hiPSC-GEPs, characterized as having a "pro-repair" astrocytic fate. Furthermore, we then investigated other factors that may influence the efficacy of the proposed stem cell therapy. We transplanted one of the hiPSC-GEP donor lines at a subacute and chronic time point, in high and low doses, and ipsilateral and contralateral locations in both aged and young mice. Once we ascertain the greatest behavioral recovery, we can assess optimal treatment conditions for inducing motor and cognitive recovery after WMS. The results obtained on this study evidence ideal conditions for transplantation location, dosage, and therapeutic window of our proposed stem cell-based therapy useful in future clinical applications of hiPSC-GEPs in neurodegenerative diseases such as WMS and VaD.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.18

Title: WITHDRAWN

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.19

Title: WITHDRAWN

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.20

Topic: C.09.Stroke

Support: NINDS R37 NS056839

Title: Bimanual training improves unimanual task performance after motor cortical infarcts in mice

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Abstract: Strokes are a major cause of severe long-term disability. Post-stroke dysfunction often results in the development of compensatory behavioral strategies that can encourage disuse contributing to persistent paretic limb dysfunction. Stroke survivors may develop greater dependence on the non-paretic side of the body to perform daily activities after stroke. Overuse of such compensatory strategies may impact post-stroke neural reorganization patterns through neuroplasticity dependence mechanisms. Studies have shown that learning compensatory ways of using both limbs together is beneficial for unimanual function on the paretic side. We hypothesize that bimanual experiences cooperate with patterns of synaptic change mediating paretic forelimb function improving unimanual performance. Mature adult C57BL/6J mice aged 4-6 months at the time of infarct are being used with equal numbers of each sex. Cranial window over forelimb area of M1 contralateral is implanted. A week later bimanual training is initially performed by 5 days. The clear plexiglas training chamber gives mice limited opportunities to use their front paws, to conquer popcorn placed on a platform outside the chamber. Subsequently, the animals were trained in the task of retrieving a single seed with their left or right paw. Photothrombotic infarct were performed in the motor cortex 2 days after behavioral training. A penetrating arteriole that supplies the motor region of the forelimb is illuminated with a 20 mW 532 nm laser. Deficits in single seed reaching performance are probed initially 2 days after photothrombotic infarct and then once a week for 7 weeks. Bimanual rehabilitation training (RT) takes place 5 days a week for 4 weeks starting 7 days after ischemia. Repeated measures ANOVA, showed that photothrombotic infarct targeting M1 cortex arteries in mice, significantly impairs the performance of an acquired skillful reaching task, significantly reducing the success of single-seed retrieval. Was observed that the impairment in the performance of these animals

persisted for 7 weeks thereafter. However, in mice that received bimanual RT for 4 weeks after infarction, the limb impaired in the same performance of the reaching task recovered to near baseline levels from the 2nd week of bimanual RT. The results showed that the effect of bimanual RT maintained the improvement in unimanual performance through week 5 but was not able to sustain the performance at weeks 6 and 7 when animals did not receive bimanual training. Therefore, bimanual rehabilitation training was shown to be able to improve the performance of paretic unimanual activity after M1 cortical infarctions in mice.

Disclosures: T.R. Da Silva: None. **V. Nemchek:** None. **T.A. Jones:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.21

Topic: C.09.Stroke

Support: NSF Grant 1915083
NSF Grant 1915065

Title: Detecting Post-Stroke Spatial Neglect and Predicting Responses to Visual Targets with an Augmented Reality-EEG System

Authors: *J. MAK[1,2,3], D. KOCANAOGULLARI[4], X. HUANG[7], M. SHIH[5], E. GRATTAN[5], E. R. SKIDMORE[5], G. F. WITTENBERG[6,2,3], S. OSTADABBAS[7], M. AKCAKAYA[4]; [1]Bioengineering, [2]Rehab Neural Engin. Labs, [3]Ctr. for Neural Basis of Cognition, [4]Electrical and Computer Engin., [5]Occup. Therapy, [6]Neurol., Univ. of Pittsburgh, Pittsburgh, PA; [7]Electrical and Computer Engin., Northeastern Univ., Boston, MA

Abstract: Spatial neglect (SN) is a common consequence of stroke that is characterized by impaired attention to contralesional stimuli. The classic gold standard assessments of pen-and-paper tests like the Behavioral Inattention Test (BIT) lack consistency and the ability to account for compensatory movements. We have developed a more objective method for detecting and assessing SN by incorporating an augmented reality (AR) head-mounted display (Microsoft Hololens) to fix the display to head movements with electroencephalography (EEG) recordings. We adapted the Starry Night Test into 1) a Clicker-Based Assessment that measures reaction times via clicker press to visual targets among irrelevant distractors, and 2) an EEG-Based Assessment that collects EEG from 16 channels while watching these targets without any motor input. Five patients with spatial neglect (SN) and five without spatial neglect (WSN) were recruited. Our first goal was to identify the spatial-spectral features that would detect neglect. EEG segments were labeled “ipsilesional” or “contralesional” depending on the stroke hemisphere of the patient and the hemispace of the target being responded to. The signal power of five frequency bands (delta (0-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-45 Hz)) was calculated at all 16 electrodes. A power ratio was calculated for each

electrode and frequency band such that the log of the power spectral density in each ipsilesional response trial was divided by the average power in the contralesional response trials. Wilcoxon rank sum tests of the ipsilesional powers, contralesional powers, and power ratios found significant differences between SN and WSN groups in the whole brain delta and theta, central-parietal alpha, frontoparietal beta, and frontal-occipital gamma. Features were lateralized in the analysis of powers and symmetric in the analysis of power ratios. Logistic regressions of the significant ipsilesional and contralesional powers were able to detect neglect with average training and testing areas under the curve (AUC) of >0.98; the analysis of the significant power ratios achieved testing and training AUCs of >0.83. Our next goal was to predict whether targets would likely be fast (observed) or slow (neglected) using common spatial patterns as the feature extraction algorithm and regularized discriminant analysis combined with kernel density estimation (RDA+KDE) for classification. RDA+KDE yielded average training and testing AUCs of >0.760. These early results suggest that our system may be able to accurately detect neglect and use EEG to predict potentially neglected locations in a user's field of view.

Disclosures: J. Mak: None. **D. Kocanaogullari:** None. **X. Huang:** None. **M. Shih:** None. **E. Grattan:** None. **E.R. Skidmore:** None. **G.F. Wittenberg:** None. **S. Ostadabbas:** None. **M. Akcakaya:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.22

Topic: C.09.Stroke

Title: Post-stroke cognitive impairment and associated brain MRI findings in acute stroke patients

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Abstract: Post-stroke cognitive impairment is a common consequence of ischemic stroke. The use of magnetic resonance imaging (MRI) analyses provides valuable information about the anatomy and pathology of the brain, which may help explain the causes of post-stroke cognitive impairment. Here, we examined associations between MRI markers and cognitive impairment in stroke patients. Patients presented with acute ischemic stroke who underwent MRI imaging and have taken neuropsychological testing within 3 months post-event were retrospectively studied. Neuroimaging markers include stroke locations, number of lesions, cerebral microbleeds (CMBs), white matter changes (WMCs), and cerebral atrophy. Global cognition and multiple

cognitive domains were assessed based on the Montreal Cognitive Assessment Tool (MoCA). The association of markers with different cognitive domains were analyzed using non-parametric statistics. Preliminary data were obtained for 34 patients (median age=64; male=61%; mean educational years=9). CMBs accounted for the largest association in global cognition (P=0.03) with significant decrease in visuospatial abilities/executive function (P=0.03), attention (P=0.03), and fluency in numbers (P=0.03). Increased amounts of CMBs also revealed further decline in visuospatial abilities (P<0.05). Stroke etiologies, locations, WMCs, and cerebral atrophy were not independent determinants of decline in any cognitive domains. With further analyses, we compared chances of having mild cognitive impairment (MCI) (MoCA scores ≤23) based on the presence of CMBs and other qualitative measures. Results showed that patients with CMBs and cortical atrophy individually increase the chances of having MCI by 60-70%, while having both lesions increase that chance to 100% (χ^2=14.09; P<0.007). Similarly, when comparing CMBs and cortical lesions, each marker individually increases the chances of having MCI to 70-80%, but having both lesions did not increase the percentage of having MCI in the acute phase of stroke (χ^2=9.5; P<0.05). In conclusion, neuroimaging markers, particularly CMBs, do confer the risk of having MCI and decline in certain cognitive domains in ischemic stroke patients. Yet, there seems to be variations in additive effects of lesions which influence cognition despite knowing that certain locations, like the cortex, guide the extent of impairment at greater degrees. These results can potentially be used as prognostic markers in acute stroke. We will continue to collect more data alongside volumetric analyses to explore and potentially map these interactions.

Disclosures: A. Kasemsantitham: None. **A. Sena:** None. **N.C. Suwanwela:** None. **C. Chunharas:** None.

Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.23

Topic: C.09.Stroke

Title: “Raisin bread sign” as a radiological feature of patients with pontine autosomal dominant microangiopathy and leukoencephalopathy

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Abstract: Pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL) is a hereditary cerebral small vessel disease (cSVD) caused by pathogenic variants in the *COL4A1* 3' untranslated region (UTR). *COL4A1* encodes a collagen type IV alpha 1 chain, one of the components of the brain vascular basement membrane. The recurrent ischemic episodes are likely to start from mid-thirties to mid-forties, and the neuroimaging feature is characterized by pontine multiple small infarctions and leukoencephalopathy. Since no suggestive signs for making a diagnosis of PADMAL during their lives have been established, it is difficult to make the decision to perform genetic analysis with *COL4A1* in some cases. We attempted to detect significant features of patients with PADMAL by analyzing the radiological findings. Two patients with undetermined cSVD and one unaffected relative in Family 1 (F1) were analyzed by whole exome sequencing and Sanger sequencing. We assessed clinicoradiological characteristics of patients in F1. Subsequently, we screened 40 clinicoradiological features of patients (N=40, age of the onset: 31- 50 years old) in the cohort of juvenile cerebral vessel disease (CVD). Sanger sequencing was performed to confirm the variants of the same gene as those in F1. We compared the genetic and radiological features of our cases to those of the previously reported cases. As a result, the variant in the *COL4A1* 3'UTR was detected within the same locus in all patients in F1 but was not detected in the unaffected relative. Multiple small infarctions and white matter hyperintensities were observed on magnetic resonance imaging in both patients, and the appearance of the pons with characteristic oval small infarctions resembled "raisin bread". Among the patients in the CVD cohort, we identified two patients who had the same radiological findings as genetically confirmed PADMAL patients in F1. Both patients harbored the variant in the *COL4A1* 3' UTR within the same locus as patients in F1. This indicates the significance of this radiological feature in the diagnosis of PADMAL. In this study, we evaluated radiological features in the pons of PADMAL patients. The neuroimaging feature characterized by oval small infarctions in the pons, for which we coined the name "Raisin bread sign", is quite important in screening the patients with PADMAL. Since our study was conducted in a small number of patients at a single hospital, further accumulation of cases is needed. However, this sign enables us to make the decision to perform genetic analysis of *COL4A1* in young patients with CVD more accurately, even when sporadic.

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Poster

625. Non-Pharmacological Approaches to Stroke Treatment

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 625.24

Topic: C.09.Stroke

Title: A Retrospective Review of COVID-19 Patients with Stroke: Experience from a Comprehensive Stroke Center

Authors: R. C. BETTS[1], L. AYARI[1], M. JOHN[1], *B. F. KIRMANI, Esq.[1,2];
[1]Texas A&M Univ. Syst. Hlth. Sci. Ctr., College Station, TX; [2]Comprehensive Stroke Ctr., CHI St Joseph Hlth., Bryan, TX

Abstract: Rationale: COVID-19 is primarily a respiratory illness but reports suggest that it may lead to hypercoagulability and thrombotic complications. The aim of this project is to review the CHI St. Joseph Health Bryan Regional Hospital Comprehensive Stroke Center experience with COVID-19-positive patients who were diagnosed with stroke in regards to risk factors, hospital course, prognosis, and outcome.Methods: This is a retrospective study to review the hospital records of COVID-19-positive patients who were diagnosed with stroke from January 1, 2020, to January 5, 2022, at the comprehensive stroke center. Subject data were acquired from electronic medical records. Approval of this retrospective analysis was given by our central CHI Health Institutional Review Board. Results: In this study, we report our preliminary retrospectively analyzed data on 15 patients who were diagnosed with stroke in the setting of COVID-19 infection. The Median age is 76. 10 patients were over 65 (67%) and 5 (33%) were under 65. There were 9 (60%) females and 6 (40%) males. 10 were white/caucasian (67%), 3 were African American (20%), and 2 were Latino (13%). 10 (67%) ischemic strokes,1 (6%) subarachnoid hemorrhage, 3 transient ischemic attacks (20%), and 1 (6%) hemorrhagic stroke were diagnosed. 8 (53%) patients presented with stroke-like symptoms as initial presentations to the hospital emergency department. 4 (27%) were diagnosed with COVID-19 prior to the admission but stroke-like symptoms prompted the hospitalization and 3 (20%) were admitted with respiratory symptoms and had a stroke in the hospital. The common comorbidities include hypertension, dyslipidemia, cardiac disease, chronic kidney disease, atrial fibrillation, and diabetes. The median National Institutes of Health Stroke Scale is 4.5 at admission with a median improvement of 0.5. The median modified Rankin Scale is 3.5 at discharge. 12 patients were discharged home or to acute rehabilitation,1 to hospice, and 2 were deceased.Conclusions: The retrospective study analysis concludes that stroke is seen in patients hospitalized with COVID-19 illness. Most strokes were ischemic and manifested as the presenting symptom for the hospital admission. This risk seems to be highest in the older population and those with pre-existing vascular risk factors.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.01

Topic: C.10. Brain Injury and Trauma

Support: ZIA-HD008966 (NICHD)

Title: Layer V projection neurons selectively undergo stress responses following mild traumatic brain injury

Authors: *M. ALKASLASI[1], A. GABLE[2], C. E. LE PICHON[3];
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Abstract: Mild traumatic brain injury (mTBI) occurs when there is sudden and rapid movement of the brain within the skull. This mild and indirect brain injury leads to a pathological cascade involving many cell types and structures in the brain, and induces injury responses including excitotoxicity, impaired axonal transport, and neuroinflammation. While the overall pathology of mTBI has been widely studied, the neuron-intrinsic responses and outcomes have not been elucidated. Here, we evaluate whether injured cortical neurons show signs of regeneration in a mouse model of mTBI. In this study, anesthetized mice were administered a unilateral closed-skull controlled cortical impact injury over the motor cortex. Mice were followed up to 10 weeks post injury to evaluate degeneration, stress response activation, neuroinflammation, and cell survival. Here, we find that layer V projection neurons (PNs) are particularly vulnerable to damage following mTBI, exhibiting axon swellings characteristic of diffuse axonal injury as well as dendrite degeneration. A subset of PNs upregulate Activated Transcription Factor 3 (*Atf3*), a transcription factor that is activated in response to axon injury. As a canonically regeneration-associated gene, expression of *Atf3* in the non-regenerative environment of the central nervous system is surprising. By permanently labeling Atf3-expressing neurons, we show that layer V PNs express markers of axon injury, upregulate pre-apoptotic genes, and are ultimately phagocytosed following mTBI. The concurrent upregulation of regenerative genes as well as pre-apoptotic genes indicates an attempt at a regenerative response that is ultimately prevented. Understanding the transcriptional cascade that is initiated in layer V PNs following mTBI may elucidate the neuron-intrinsic barriers to CNS regeneration.

Disclosures: **M. Alkaslasi:** None. **A. Gable:** None. **C.E. Le Pichon:** None.

Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.02

Topic: C.10. Brain Injury and Trauma

Support: NIH R01NS093992
NIH R01NS113516
NIH R21AG066496
Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation
Semmes Foundation

Title: Molecular mechanisms of aberrant adult-born granule cell migration

Authors: *G. CHANGARATHIL[1], P. VARMA[1], A. ADEYEYE[1], M. COPPIN[1], Z. LYBRAND[2], J. HSIEH[1];
[1]Univ. of Texas at San Antonio, San Antonio, TX; [2]Texas Woman's Univ., Denton, TX

Abstract: Studies show seizure induced aberrant hippocampal neurogenesis contribute to the development of chronic seizures. Our lab reported that 2-week-old (2w) adult-born granule cells (abGCs) in the mouse dentate gyrus display elevated Ca^{2+} activity after pilocarpine (pilo) induced seizures compared to sham. Moreover, this amplified Ca^{2+} activity is reduced by chemogenetic DREADDs/hM4Di silencing in pilo treated mice. The molecular mechanisms by which elevated Ca^{2+} activity drives aberrant hippocampal neurogenesis and, in turn, epileptogenesis is unknown. Hence, we hypothesize that early activity in abGCs alters Ca^{2+} mediated gene expression that promote aberrant maturation, including ectopic migration of abGCs associated with spontaneous recurrent seizures. RNA-sequencing analysis from FACS sorted 2w old abGCs after pilo treatment and hM4Di silencing revealed differentially expressed genes, i.e., gene expression downregulated with pilo treatment and upregulated with hM4Di silencing, among which *Timp3* was one of the most significantly differentially expressed genes. Because *Timp3* is widely reported to regulate migration and invasion of tumor cells by modifying matrix metalloproteinases, we hypothesized that seizure induced changes in the expression of *Timp3* may play a role in controlling aberrant abGC migration. First, we confirmed in RNAscope analysis that *Timp3* is expressed in abGCs which is downregulated in pilo treated mice. To determine the role of *Timp3* in aberrant abGC development, we used the LXR agonist T0901317 reported to knockdown its expression. Treatment of mice with T0901317 for 2w resulted in an increased number of hilar ectopic abGCs and granule cell dispersion. These results suggest that alterations in Ca^{2+}activity within 2w old abGCs after pilo leads to changes in gene expression, including *Timp3,* which could play a role in aberrant migration of abGCs. Further experiments are being performed to address whether *Timp3*-mediated aberrant migration is sufficient for spontaneous recurrent seizures. Our work also demonstrates a new way to manipulate potential aberrant gene regulatory pathways and define the functional role of our top candidate gene - *Timp3* - in aberrant abGC neurogenesis.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.03

Topic: C.10. Brain Injury and Trauma

Support: TWU foundation grant FY2022 Chancellor's Research Fellows

Title: Characterizing single cell RNA sequencing of a cerebral organoids in a blast traumatic brain injury model

Authors: *N. YASIN, T. VU, A. GOMEZ, Z. LYBRAND;
Texas Woman's Univ., Denton, TX

Abstract: Neurodegeneration is a characteristic of many incurable diseases increasing in prevalence, such as Alzheimer's disease and Chronic Traumatic Encephalopathy (CTE). CTE results from repetitive mild traumatic brain injury (mTBIs), commonly experienced by athletes and soldiers. Limited understanding of the mechanism due to the lack of in-vitro models of mTBIs prevents adequate diagnosis, monitoring, and treatment of CTE. Previously, we characterized parameters of Traumatic Brain Injury (TBI) in vitro using human cerebral organoids. Using a benchtop blast chamber, we exposed organoids to pressure forces common in TBI. In this study, we used parameters found in the mTBI range to study the transcriptomic changes of separate cell populations in brain organoids to understand potential mechanisms of injury-induced neurodegeneration better. Cerebral brain organoids were generated from human-induced pluripotent stem cells using a dual SMAD inhibition protocol to generate pallial and subpallial spheroids. Spheroids were fused to assemble a cerebral organoid and grown for six months. Fully assembled organoids were loaded into a benchtop blast chamber and exposed to a high-frequency pressure wave. Control organoids were loaded into the chamber without exposure to pressure waves. Three organoids from the no blast control and three organoids from the blast group were gently dissociated. A total of 10,000 cells per group were prepped for single-cell GEMs and cDNA library preparation per the manufacturer's protocol before sequencing. Cell Ranger Aggr v7.0.0 was used for single-cell analysis to generate aggregated FASTQ files aligned to the GRCh38 human reference genome. Preprocessing steps were performed using the Python toolkit, Scanpy, for analyzing single-cell data. Cells with fewer than 2,000 genes expressed and genes expressed in <10% were filtered. Cells expressing greater than 10% mitochondrial genes expressed were additionally filtered. The final analysis includes 3385 cells in the control group and 4257 cells in the blast group. Eleven unique clusters were identified using principal component analysis and UMAP. Three separate neuronal clusters were (SYN1, STMN2, GRIA4, and GABRA2). Additionally, astrocytes (GFAP, S100B, AQP4), progenitor cells (TOP2A, BIRC5, PAX6, MK167), oligodendrocytes (MBP, MAL), and radial glial (NES, GFAP, HES1, HOPX). For each cluster, differential gene expression analysis between cells from the control group and cells from the blast group. Preliminary analysis indicates pathways involved in protein degradation via ubiquitination and integral membrane proteins are disrupted in response to blast parameters.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.04

Topic: C.10. Brain Injury and Trauma

Support: Citizens United for Research in Epilepsy (CURE)
R01 NS112350 (NHV)
R01 NS112308 (RD)

Title: Pharmacological inhibition of the inflammatory receptor CCR2 relieves the immediate deleterious consequences of status epilepticus

Authors: *N. H. VARVEL[1], C. ALEMAN-RUIZ[2], W. WANG[2], R. J. DINGLEDINE[3]; [1]Emory Univ., [2]Emory Univ., Atlanta, GA; [3]Emory Univ. Sch. Med., Emory Univ. Sch. Med., Atlanta, GA

Abstract: Generalized status epilepticus (SE) triggers a robust neuroinflammatory response involving reactive astrocytosis, activation of brain-resident microglia, and brain infiltration of CCR2+ monocytes. Multiple lines of evidence indicate that quenching SE-induced neuroinflammation can alleviate the adverse consequences of SE, including neuronal damage and cognitive impairments. Our recent findings show that blocking monocyte brain entry after SE, via global *Ccr2* KO, rescues several SE-induced adverse effects including blood-brain barrier (BBB) erosion and neuronal damage while enhancing weight regain. The goals of the present study were to determine if CCR2 antagonism with a small molecule after SE blocks monocyte brain entry, dampens brain inflammation, and provides neuroprotection three days after SE. Eight to ten-week-old male *Ccr2*$^{+/rfp}$ heterozygous mice maintained on the C57BL/6CR inbred genetic background were subject to intraperitoneal injection of kainic acid (KA), scored for seizure severity, weight recovery and nest building capability, then sacrificed three days post-SE. Surviving mice were randomized into CCR2 antagonist (n=19) and vehicle (n=20) groups. The CCR2 antagonist (100mg/kg), or vehicle, was administered 24- and 48- hours post-SE via oral gavage, the dose timing based on our observation that monocyte entry into the brain begins around two days after SE. Mice subject to the CCR2 antagonist displayed faster weight recovery between one- and three-days post-SE and enhanced ability to build a nest on the third day after SE when compared to their vehicle-treated controls. CCR2 antagonism limited monocyte recruitment to the hippocampus and reduced numbers of Iba1+ macrophages. The mRNA levels of inflammatory mediators were depressed by 47%, and the glial markers were reduced by 30% in mice treated with the CCR2 antagonist compared to controls. Astrocytosis, but not microgliosis, was reduced in four brain regions. Neuroprotection was observed in the hippocampus, and erosion of the BBB was lessened in mice subject to the antagonist. Our findings provide proof-of-concept that CCR2 antagonism after SE can alleviate multiple adverse effects, and identify CCR2+ monocytes as a viable therapeutic target.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.05

Topic: C.10. Brain Injury and Trauma

Support: NS111378
NS117148
NS050465
NS116838

Title: Thyroid hormone (T4) attenuates peripheral TBI pathology

Authors: *M. KHANDELWAL, G. KRISHNA, Z. YING, F. GOMEZ-PINILLA;
Univ. of California, Los Angeles, CA

Abstract: Traumatic brain injury (TBI) is a common burden occurring in sports, military and domestic accidents affecting 10 million people annually worldwide. In TBI, since brain is the primary site of injury, most of the research has been centered cerebrally. However, injury to the brain elicits a rapid peripheral response in which prolonged metabolic disturbance and hyperinsulinemia can cause serious outcomes involving the brain-gut interaction. Additionally, higher consumption of fructose present in soft drinks and processed foods can also elicit serious metabolic dysfunction including metabolic syndrome, insulin resistance and obesity in periphery. Our study focusses on candent questions that are poorly explored. 1. How brain injury stimulus affects peripheral tissues *via* brain-liver axis? 2. Does alterations in brain pathogenesis result in liver steatosis? 3. Do the effects of consumption of a high fructose rich diet, normally metabolized in the liver, influence the action of TBI on the liver? 4. Does thyroid hormone activation can help in regulating lipid metabolism and insulin signaling in liver? In particular, lack of knowledge about metabolic disturbances in peripheral tissues in TBI survivors is a big concern. It is important to have deeper knowledge about the impact of TBI pathology on the metabolic master regulator liver tissue specially in individuals consuming a high fructose diet. In our study we performed fluid percussion injury (FPI) in SD rats with and without high fructose ingestion, and a group of rats was treated with thyroid hormone (T4). T4 hormone is a strong metabolic modulator affecting many tissues in the body and brain. Our results showed that T4 treatment was effective in regulating lipid metabolism by reducing *de novo* lipogenesis, lipid accumulation, lipogenic enzymes (ACC1, AceCS1, FAS), lipid peroxidation compared to the diseased controls. Moreover, T4 treatment improved the insulin signaling in TBI rats with/without fructose ingestion compared to their respective diseased controls. Additionally, hepatic inflammation was also reduced with T4 treatment as compared to their respective controls. In conclusion, T4 hormone treatment was highly effective in the regulation of peripheral TBI pathology, and results suggest that T4 may be specially effective in individuals who consume high fructose. Thus, modulating levels of T4 could be a promising approach for treating TBI pathology in individuals with/without consuming high fructose diet.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.06

Topic: C.10. Brain Injury and Trauma

Support: ONR Grant (N000141812494)

Title: Neurodegeneration and Neuroinflammation in Cortical Neurospheroids Subjected to Centrifugation-induced Compressive Injury

Authors: *R. D. GONZALEZ-CRUZ[1], Y. WAN[2], D. ALAM EL DIN[1], D. CALVAO[3], W. K. RENKEN[1], H. KESARI[2], C. FRANCK[4], D. HOFFMAN-KIM[1];
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Abstract: Sustained compressive injury to the brain typically occurs after head injuries that generate damaging intracranial pressures such as those caused by intracranial hemorrhages, tissue swelling, or a heavy object crushing the brain. Most compressive brain injury cellular models have looked at the effects of compressive tissue damage in the context of traumatic brain injury, which is triggered by tissue deformations happening over very short time spans. However, few in vivo and in vitro studies have examined the effects of sustained compressive injury on neuronal cell death, neurodegeneration, and neuroinflammation. Here, we present an *in vitro* model of sustained compressive neuronal injury and neuroinflammation in which we compress cortical neurospheroids via centrifugation. Spheroids were made from isolated, neonatal rat cortical cells that were seeded at 4000 cells/spheroid and incubated for 14 days *in vitro*. A subset of spheroids was centrifuged once at angular velocities of 0, 209, or 419 rad/s for 2 minutes using a cell culture-grade centrifuge. Using finite element modeling, we found that spheroids centrifuged at 209 and 419 rad/s experienced compressive strains of 10% and 19%, respectively. We also examined cellular injury post-centrifugation in living control and injured spheroids via LIVE-DEAD assay and Hoechst 33342 nuclear staining. Neuronal degeneration and astrogliosis were assessed via confocal imaging of β_3-tubulin and glial fibrillary acidic protein (GFAP) immunostaining at 0, 2, 8, and 24 hours post-centrifugation injury. Microglia activation was assessed via confocal imaging of Iba1 immunostaining in a subset of fixed spheroids at 1, 3, 5, and 7 days post-injury. Centrifuged spheroids exhibited higher DNA damage than control spheroids 24 hours post-injury experiments ($p < 0.05$). Qualitative assessment of β_3-tubulin networks showed increasing degradation of microtubules over time with increasing angular velocity. Immunostaining of GFAP and Iba1 in both control and injured spheroids showed increased astrocyte reactivity and microglial activation in injured spheroids ($p < 0.05$). However, we noticed astrocyte reactivity occurred within the first 24 hours post-injury while microglia activation was observed at later time points. Our findings show that neuronal injury and reactive gliosis can occur as a result of sustained compressive tissue strains. This experimental compressive injury model provides an *in vitro* platform to examine injury and inflammatory cellular thresholds to gain insights into injury mechanisms that could be targeted with therapeutic strategies.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.07

Topic: C.10. Brain Injury and Trauma

Support: NIH Training Grant 5T32 NS077889, Neurobiology of CNS Injury and Repair
Kentucky Spinal Cord and Head Injury Research Trust Fellowship Funds

Title: Traumatic brain injury induces acute intestinal permeability, increased colon hypoxia, and subacute changes to the gut microbiome of mice

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Abstract: Traumatic brain injury (TBI) triggers not only neurovascular and glial changes within the brain, but also systemic responses that can include gastrointestinal (GI) dysfunction. Even in the absence of polytrauma, brain-injured individuals may suffer intestinal inflammation or ulceration, fecal incontinence, or GI-related mortality. Research across a wide spectrum of disorders now implicates the gut microbiome as a key regulator of disease and suggests that dysregulation of the gut microbiota affects brain function. Recent findings associate TBI with altered fecal microbial diversity, but little is known about the timeline of these changes and their relation to gut dysfunction or pathology. Examination of hematoxylin/eosin stained intestinal tissue from mice receiving sham or controlled cortical impact (CCI) TBI (n=3-8/injury group at 7 timepoints: 4hr-4wks) revealed no overt damage in the ileum or colon. To interrogate intestinal permeability, FITC-Dextran (4kda) was orally administered prior to euthanasia (n=6-8/injury group, 4 timepoints: 4hr-3d). Quantification of serum fluorescence revealed an increase in permeability at 4hr after CCI (p=0.0067 compared to sham). To determine a timeline of post-TBI gut microbiome changes, fecal samples were collected prior to and after sham or CCI injury (n=6-7/group, 6 timepoints: 1d-4wk) for 16s gene sequencing. The phylum *Verrucamicrobiota* was differentially abundant in CCI mice at 1, 2, and 3d postinjury (ANCOM-BC; q<0.05). qPCR was conducted to identify the *Verrucamicrobiota* species as *Akkermansia Muciniphila*. This species resides in and regulates the intestinal mucous layer. Quantification of Alcian blue staining to detect mucin-producing goblet cells, however, revealed no differences in response to TBI. Because *Akkermansia Muciniphila* increases under hypoxic conditions to promote intestinal wound healing, we assessed GI hypoxia at 1d and 3d after CCI using pimonidazole-HCl administered prior to euthanasia to label hypoxic tissue (n=6-7/Injury group, n=11 sham). An increase in colon hypoxia was observed at 3d following CCI compared to sham controls (p=0.0305). Our findings suggest an acute GI disturbance and an increase of beneficial bacteria suggesting a potential compensatory response to systemic stress after TBI.

Disclosures: A.J. DeSana: None. **T.A. Barrett:** None. **K.E. Saatman:** None.

Poster

626. Traumatic Injury

Location: SDCC Halls B-H

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Program #/Poster #: 626.08

Topic: C.10. Brain Injury and Trauma

Support: DOD EP200042
NIH F31NS124290-01
R01NS069861
R01NS097750

Title: Contribution of toll-like receptor 4 : matrix metalloproteinase-9 signaling to altered network excitability and synaptic plasticity in traumatic brain injury

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Abstract: Traumatic Brain Injuries (TBI) often trigger a robust immune response characterized by the activation of inflammatory mediators that subsequently result in cell death, altered neuronal excitability, memory deficits and promotes epileptogenesis. We recently identified a key role for Toll-like receptor 4 (TLR4), an innate immune receptor, in augmenting posttraumatic network excitability. Importantly, pharmacological inhibition of TLR4 within the first 48 hours after TBI effectively reduced epileptogenesis and memory deficits in rodents. Here we examined whether TLR4 signaling recruits Matrix Metalloproteinase-9 (MMP-9), a potent Zn+ activated endopeptidase critically involved in circuit remodeling and plasticity, and if the TLR4:MMP-9 signaling axis alters neuronal excitability and synaptic plasticity in the hippocampal dentate gyrus (DG) after concussive brain injury. Rats (p24) subjected to moderate lateral Fluid Percussion Injury (l-FPI) or sham injury were treated with antagonists of TLR4 (CLI-095, 0.5mg.kg,i.p.) or MMP-9 (SB-3CT, 50mg.kg,i.p.) or vehicle 30min to 24hrs post-injury and examined at 48 hrs for MMP-9 activity and at one week for changes in DG excitability *in vivo*. Long-term changes in synaptic inputs to DG Granule Cells (GC) were examined six weeks post injury. *In situ* zymography revealed an increase in MMP-9 activity 48 hrs after l-FPI, which was reduced by CLI-095 treatment (n=4 per group). In urethane anesthetized rats, responses to activation of perforant path inputs to DG *in vivo* revealed an increase in excitability and impaired long-term potentiation one week after l-FPI, which were reduced by treatment with CLI-095 or SB-3CT (n= 4-5 per group). Ongoing studies examining GC synaptic inputs will determine if early post-injury TLR4 inhibition can mitigate long-term changes in synaptic inputs after TBI. Together, our results provide the first evidence of TLR4 mediated increase in MMP-9 activity after TBI and identify a role for TLR4:MMP-9 signaling in altered dentate excitability and plasticity following TBI.

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Poster

626. Traumatic Injury

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Program #/Poster #: 626.09

Topic: C.10. Brain Injury and Trauma

Support: WP911F-17-2-0222

Title: Changes in Dendritic Spine Morphology Within the Hyperacute Phase Following Impact in an Ex Vivo Porcine Brain Model

Authors: *B. HOFFE[1], G. KANG[2], R. BANTON[3], T. PIEHLER[3], O. PETEL[2], M. R. HOLAHAN[1];
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Abstract: Dendritic spines are responsible for relaying excitatory input and regulating signals between neurons. Their morphology can give insight into both synaptic function and dysfunction. Excitotoxicity, brought on by brain injury, results in synaptic dysfunction, as intracellular Ca^{2+} concentrations increase. This increase in intracellular Ca^{2+} over-activates various intracellular kinases and proteases responsible for microtubule stabilization. Previous work in our lab has shown that after a drop impact, there was a marked decrease in staining of the microtubules stabilizing protein MAP2 specifically within the apex of the sulcus. In our current work, we explored whether changes in spine morphology were associated with our impact model (*ex vivo* pig brains) within the hyperacute phase post-brain injury (minutes to hours). The Golgi-Cox method was used to visualize dendritic spine morphology. Following impact, coronal slabs of porcine brain tissue were placed into Golgi-Cox solution for 2 weeks. Cortical pyramidal neurons within the sulcus were reconstructed. One hour after impact, there was a decrease in thin and stubby type spine densities with a concomitant increase in the proportion of mushroom-type spines. There was also a decrease in the total spine density on the basal dendrites. Given the short time-frame following impact, the increased proportion in mushroom-type spines could be due to stronger synaptic connectivity, as well as the maladaptive use of synaptic mechanisms involved in excitatory signaling promoting an overall excitatory state of the synapse. The favoring of mushroom-type spines could give insight into the synaptic mechanisms and secondary excitotoxic conditions involved after brain injury.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.10

Topic: C.10. Brain Injury and Trauma

Support: ONR, Code 34

Title: Traumatic brain injury model in vitro to assess critical mechanical thresholds of neural network signal disruption

Authors: *J. SERGAY, L. SUMMEY, A. HAI, C. FRANCK;
Univ. of Wisconsin - Madison, Madison, WI

Abstract: Traumatic brain injury (TBI) primarily involves mechanically applied injury that can alter cellular function in the brain and cause long lasting, adverse cognitive effects. Brain functions rely on proper intercellular electrical signal propagation vital for communication between the brain and other target organs, which may be disrupted following injury. Most studies examining these effects lack assays for simultaneously controlling and measuring the cellular mechanics of injury in parallel with imaging network wide, single-neuron level signaling. In this study, we aim to establish a distilled *in vitro* protocol used to create a deterministic link between the mechanical deformations leading to trauma and the resulting electrophysiological changes. Neuronal cultures are established on specialized “dogbone-shaped” polydimethylsiloxane (PDMS) substrates made with a novel molding technique on silicon wafers. The PDMS is stretchable and nearly linearly elastic, allowing for uniform strain application throughout the network. Our assay uses primary cortical neural cells from P0 rats that are seeded on the PDMS platform treated with poly-d-lysine (0.1 mg/ml) and laminin (0.4 mg/ml) at a density of 2,500 cells/mm^2. A custom-built tension device applies uniaxial strain to the PDMS at a prescribed strain magnitude and rate. The device was validated by stretching speckled PDMS dogbones and tracking the stretch with a digital-image-correlation algorithm. The device was found to accurately apply programmed strains between 0% to 200% and strain rates between 0 s^{-1} to 225 s^{-1}, closely mimicking the strain rates occurring in the brain during impact and blast TBI. Significant neuronal cell signaling disruption is determined by capturing spontaneous activity using a standard calcium (Ca^{2+}) probe Fluo-4 AM. Ca^{2+} dynamics are quantified by extracting spike frequencies and Ca^{2+} fluorescent intensities from 90 second time lapses captured at 20 Hz. This protocol can allow for the assessment of the first strain and strain rate thresholds for significant neural cell signaling perturbation quantified by changes in calcium activity. The broader implication of the protocol provides improved quantitative characterization of the cellular response to specific mechanical TBI conditions that will inform computational, clinical, and safety models to improve tailored diagnosis and early treatment for TBI.

Disclosures: J. Sergay: None. **L. Summey:** None. **A. Hai:** None. **C. Franck:** None.

Poster

626. Traumatic Injury

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Program #/Poster #: 626.11

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01 NS092552

Title: Dentate granule cell birth date-specific upregulation of feedback inhibition in a mouse model of traumatic brain injury

Authors: *Y.-J. KANG[1,2], S.-H. LEE[1,2,3], J. A. BOYCHUK[4,3], C. R. BUTLER[4], A. JURAS[2], R. A. CLOYD[4], B. N. SMITH[1,2,3,4,5];
[1]Biomed. Sci., Colorado State Univ., Fort Collins, CO; [2]Neurosci., [3]Epilepsy Res. Ctr., [4]Physiol., [5]Spinal Cord and Brain Injury Res. Ctr. (SCoBIRC), Univ. of Kentucky, Lexington, KY

Abstract: Posttraumatic epilepsy (PTE) and behavioral comorbidities frequently develop after traumatic brain injury (TBI). Aberrant neurogenesis of dentate granule cells (DGCs) after TBI may contribute to the synaptic reorganization that occurs in PTE, but how neurogenesis at different times relative to the injury contributes to feedback inhibition and recurrent excitation in the dentate gyrus is unknown. Thus, we examined whether DGCs born at different postnatal ages differentially participate in feedback inhibition and recurrent excitation in the dentate gyrus using the controlled cortical impact (CCI) model of TBI. Both sexes of transgenic mice expressing channelrhodopsin2 (ChR2) in postnatally born DGCs were used for optogenetic activation of three DGC cohorts: postnatally early born DGCs, or those born just before or after CCI. We performed whole-cell patch-clamp recordings from ChR2-negative, mature DGCs and parvalbumin-expressing basket cells (PVBCs) in hippocampal slices to determine whether optogenetic activation of postnatally born DGCs increases feedback inhibition and/or recurrent excitation in mice 8-10 weeks after CCI and whether PVBCs are targets of ChR2-positive DGCs. In the dentate gyrus ipsilateral to CCI, activation of ChR2-labeled DGCs born before CCI produced increased feedback inhibition in ChR2-negative DGCs and increased excitation in PVBCs compared to those from sham controls. In sharp contrast, this upregulated feedback inhibition was absent in DGCs born early in life or after CCI. Surprisingly, ChR2-positive DGC activation rarely evoked recurrent excitation in mature DGCs from any cohort. These results suggest that DGC birth date-specific increased feedback inhibition in of DGCs may contribute to altered excitability after TBI.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.12

Topic: C.10. Brain Injury and Trauma

Support: Brockman Foundation

Title: Untargeted Metabolomics Analysis of Mouse Cerebral Cortex After Multimodal Traumatic Brain Injury Shows Changes in Sphingolipid Levels

Authors: *S. CORELLA[1,2,4,5,3], M. M. DHAR[1,2,4,5,3], K. CHAUBEY[1,2,4,5,3], K. Z. FRANKE[1,2,4,5,3], C. J. CINTRON[1,2,4,5,3], A. A. PIEPER[1,2,4,5,3];
[1]Pathology, [2]Psychiatry, [3]Inst. for Transformative Mol. Med., Case Western Reserve Univ. Sch. of Med., Cleveland, OH; [4]Harrington Discovery Inst., Univ. Hosp., Cleveland, OH; [5]Geriatric Res. Educ. and Clin. Ctr. (GRECC), Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH

Abstract: In the United States, about 2.5 million cases of traumatic brain injury (TBI) are diagnosed annually. TBI has recently been recognized as a chronic neurodegenerative condition, with symptoms persisting and progressing for years after initial injury. Moreover, a single TBI increases the risk of developing Alzheimer's disease (AD) by 50% and accelerates the onset of cognitive decline in AD by 3-4 years. Unfortunately, the mechanistic basis for progression of neurodegeneration after TBI is poorly understood. Thus, there are still no neuroprotective treatments that can attenuate or stop the process. To address this gap in knowledge, we are measuring differential metabolite patterns, in an unbiased manner, following a multimodal TBI in mice. This model induces a complex and rigorously reproducible head injury that includes components of global concussion, acceleration / deceleration injury, and early blast wave exposure. It produces a reliable pattern of neurodegeneration, cognitive impairment, and peripheral metabolomic changes that mimic human TBI. We hypothesized that TBI would significantly alter cortical metabolites, and that pharmacologic protection of the brain with the neuroprotective compound P7C3-A20 would normalize at least some of the TBI-induced aberrations. We used four groups of 8-week-old male mice: (1) sham-vehicle, (2) sham-A20, (3) TBI-vehicle, (4) TBI-A20. In TBI groups, cortical sphinganine levels were significantly reduced following injury. When the brain was protected with P7C3-A20, however, levels of sphinganine were restored to sham-vehicle levels. Given that sphinganine is a ceramide precursor molecule in de novo sphingolipid synthesis, our findings show that TBI impacts the process of de novo sphingolipid synthesis. Furthermore, our results suggest that enhancing de novo sphingolipid synthesis may be therapeutic, as de novo sphingolipid synthesis allows for the restoration of complex sphingolipids and ultimately cortical repair. An immediate goal in our study is to validate our non-targeted metabolomics findings in an additional cohort of animals, and then to model this system further in vitro to methodically investigate the underlying biochemistry. Long-term, we hope to identify differential metabolomic profiles between sham and TBI animals that help clarify our understanding of the injury mechanisms of this clinical condition. We are also conducting analogous studies in the blood after TBI to understand the contribution of peripheral biology to chronic neurodegeneration and potentially identify novel biomarkers of TBI as well.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

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Program #/Poster #: 626.13

Topic: C.10. Brain Injury and Trauma

Support: RAC Fellowship, Children's Hospital of Pittsburgh, UPMC Health System
NIH R01-NS106925
NIH R21-NS115440
The Pittsburgh Foundation Walter L. Copeland Fund

Title: Hippocampal CA1 dendritic spine characterization and GluA1 expression 2 weeks after controlled cortical impact in rats

Authors: *S. E. SVIRSKY, H. YOON, J. HENCHIR, S. W. CARLSON, C. E. DIXON; Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Extensive effort has been made to study the role of synaptic deficits in cognitive impairment after traumatic brain injury (TBI). The dendritic spine is a dynamic structure, which functions as the anatomical locus of synaptic plasticity and underlies learning and memory. This study examined the effect of controlled cortical impact (CCI) on hippocampal CA1 dendritic spine density and morphology, along with protein expression of hippocampal α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA1 sub-unit, a protein integral to synaptic plasticity. Adult, male Sprague Dawley rats (275-300g, 3 animals per group, 2-3 neurons per animal) received either CCI (2.5mm deformation, 4m/s) or control/Sham surgery. 2 weeks post-injury, brains were processed for Golgi staining, z-stacks were imaged on a Nikon confocal microscope and spines were counted and classified using Neurolucida 360 software. No change in spine density was observed between groups. However, there was a trending decrease in the number of "thin" and "mushroom" spines on apical dendrites (Student's t-test, $p=0.0968$, $p=0.0584$) and a significant decrease in "mushroom" spines on basal dendrites ($p<0.05$) after CCI compared to Sham. There was a significant increase in number of "stubby" spines on apical dendrites after CCI ($p<0.05$). Protein expression of GluA1 was measured by western blot in ipsilateral hippocampal synaptosomes (10 animals per group). There was a significant decrease in GluA1 expression after CCI compared to Sham ($p<0.0001$). In conclusion, CCI significantly alters CA1 dendritic spine morphology and GluA1 expression 2 weeks post-injury, reflective of cognitive deficits previously observed at this time-point. This metric may be used to evaluate future therapeutic studies targeting synaptic plasticity deficits after TBI.

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Poster

626. Traumatic Injury

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Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS1108098
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Title: Sex-dependent cognitive dysfunction following repeated mild TBI in adolescent animals may be dependent on alterations in acetylcholine and corticotropin releasing factor expression

Authors: *T. A. MCCORKLE, R. RAGHUPATHI;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Sports-related concussions (a subset of mild TBI) are a leading cause of long-term cognitive deficits in adolescents. Moderate TBI and chronic stress in animals lead to impairments in hippocampal-dependent memory due to alterations in expression of choline acetyltransferase (ChAT) or corticotrophin releasing factor (CRF) within the medial septum (MS). Previously, we had reported that repeated mild injuries in adolescent male and female rats resulted in spatial memory deficits at 1-and-4-weeks post-injury in the novel object location task; only male brain-injured animals exhibited significant deficits at 1 week whereas both male and female brain-injured animals showed impairment at 4 weeks. We hypothesized that disrupted cholinergic transmission between the MS and hippocampus may be the mechanistic basis for these deficits, and that CRF expression in the amygdala regulated ChAT expression in the MS. Following behavioral assessment at each time point, rats were sacrificed for quantitative real-time PCR and immunohistochemistry. Our data show that there is a decrease in ChAT immunoreactivity in the MS of male brain-injured animals at 1-and-4-weeks, but fewer ChAT(+) cells were observed in female brain-injured animals only at the 4 week time point. CRF immunoreactivity within the MS was only increased in female brain-injured animals, and immunoreactivity in the amygdala showed no change in any brain-injured animal. However, CRF mRNA within the amygdala was increased 1-and-4-weeks post-injury only in male brain-injured animals. These results provide novel sex-dependent associations between ChAT and CRF expression and cognitive impairments post-injury, offering further insight into a potential mechanism of action.

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Poster

626. Traumatic Injury

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Program #/Poster #: 626.15

Topic: C.10. Brain Injury and Trauma

Support: NS111378
NS117148
NS050465
NS116838

Title: The BDNF mimetic R-13 attenuates TBI pathogenesis using PI3K/Akt signaling and mitophagy

Authors: *P. THAPAK[1], G. SMITH[2,3], L. YING[1], A. PAYDAR[2,3], N. HARRIS[2,3], F. GOMEZ-PINILLA[1,3];
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Abstract: The BDNF mimetic R-13 attenuates TBI pathogenesis using PI3K/Akt signaling and mitophagy Pavan Thapak[1], Gregory Smith[2,3], Lily Ying[1], Afshin Paydar[2,3], Neil Harris[2,3], and Fernando Gomez-Pinilla[1,3]1.Dept. Integrative Biology and Physiology, UCLA, Los Angeles, CA 2.Neurosurgery, UCLA David Geffen School. of Medicine, Los Angeles, CA3.UCLA Brain Injury Research Center, Los Angeles, CA
Background: Traumatic brain injury (TBI) is a major neurological burden globally and escalates psychiatric disorders. The R13 is a TrkB agonist molecule much smaller and more effective than BDNF with great potential to treat neurodegenerative disorders, which capacity has not been shown in the TBI pathology. **Methods:** Sprague Dawley rats both male and female were injured by lateral fluid percussion injury (FPI; X= -4.5mm from bregma, Y= 2.5mm midline to left), and R13 (7.25 mg/kg, i.p) and vehicle were administered 24 hr post-injury for 7 consecutive days. Magnetic resonance imaging (MRI) was performed after injury on the 1st and 7th days. Memory and anxiety-like behaviors were assessed one-week post-TBI and protein levels were measured in the ipsilateral hippocampus using quantitative western blots.**Results:** Male and female rats exposed to FPI showed a significant reduction in spatial memory and anxiety-like behavior which was counteracted by R13 treatment. Deficits in FC and mean diffusion occurred in TBI rats compared to shams while R13 significantly improved FC and MD. In addition, injured animals showed a significant reduction in the protein expression of p-TrkB, p-PI3K, p-AKT, GluR2, PSD95, PINK, Parkin, and LC3 in the ipsilateral hippocampus as compared to sham animals, which were mitigated by R13 intervention. **Conclusion:** This study showed that R13 counteracted FC deficits in circuits related to altered cognitive impairment through BDNF/TrkB/PI3K signaling and mitophagy in the hippocampus of post-TBI rats. Overall, these findings delineate the neuroprotective potential of R13 against TBI.

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Poster

626. Traumatic Injury

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Topic: C.10. Brain Injury and Trauma

Support: CIHR Grant 385495

Title: Senolytic treatment in a murine model of mild traumatic brain injury with senescent-like neurons

Authors: *N. SCHWAB[1,2], D. TASKINA[1], Y. JU[1], B. INNES[3], G. BADER[3], L.-N. HAZRATI[4]; [1]Neurosci. and Mental Hlth., SickKids Res. Inst., Toronto, ON, Canada; [2]Lab. Med. and Pathobiology, [3]Dept. of Mol. Genet., Univ. of Toronto, Toronto, ON, Canada; [4]Dept. of Pediatric Lab. Med., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Mild traumatic brain injury (mTBI) can have long-term consequences on brain health, including physical and mental symptoms alongside an increased risk of neurodegenerative disease and dementia later in life. The molecular mechanisms driving these changes remain unclear, and there are currently no effective therapies for mTBI patients. In this study, we have used a closed-skull model of repeated mTBI in sex-balanced groups of C57BL/6 mice and sham procedures to investigate the role of cellular senescence in mTBI pathophysiology. A week following mTBI procedures, male and female injured mice were cognitively impaired, demonstrated by impaired performance in the Morris Water Maze compared to sham animals, and displayed behavioral disinhibition and recklessness, demonstrated by spending more time in the light chamber in the light dark box task. The latter deficit was predominantly seen in female mice, indicating a sex difference in behavioral outcomes. Cortical and hippocampal tissue revealed increased DNA damage in the form of double-strand breaks, oxidative damage, and accumulation of R-loops at one week post injury in injured animals compared to shams. Similarly p16 and p21, markers of cellular senescence, were increased at this timepoint in injured mice compared to shams, with sex-specific differences in expression. The DNA damage response showed clear sex discrepancies with female mTBI mice upregulating BRCA1 and male mTBI mice reducing BRCA1 expression and instead upregulating RAD51. Single cell RNA sequencing and subsequent gene set enrichment analysis (GSEA) revealed a senescent gene signature in both glial and neuronal cell types, including the senescent-associated secretory phenotype, anti-apoptotic mechanisms, metabolism shifts, and cell cycle checkpoints. A second cohort of mice were systemically treated with the senolytic drug ABT-263, which selectively targets senescent cells for apoptosis, one week post-injury, which was shown to significantly improve performance in the Morris Water Maze, reduce the DNA damage response, and reduce cellular senescence. Our study provides compelling evidence that cellular senescence is a pathophysiological mechanism driving brain dysfunction after mTBI, and that targeting senescence therapeutically may be beneficial. However, sex differences in DNA repair strategies indicate that personalized treatment targets which consider sex may be needed for clinical translation.

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Poster

626. Traumatic Injury

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Topic: C.10. Brain Injury and Trauma

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Title: Long-term alterations of neuronal activity in freely-behaving mice after a single juvenile concussion

Authors: *C. J. DUBOIS[1], L. HIPPAUF[1], R. ROULAND[1], A. OBENAUS[2], J. BADAUT[1,3]; [1]CRMSB UMR 5536, Univ. de Bordeaux, Bordeaux, France; [2]Pediatrics, Univ. of California, Irvine, Irvine, CA; [3]Basic sciences, Loma Linda Univ., Loma Linda, CA

Abstract: Traumatic brain injuries (TBI) represent the most frequent cause for paediatric emergency medical care visits. While about 20% of the children suffering mild TBIs will develop long-term cognitive and emotional impairments, there is no specific treatment and the underlying cellular and molecular mechanisms remain poorly understood.
We aimed to determine how neuronal activity is affected over time into adulthood after a juvenile mild TBI (jmTBI) at rest and during behavioural tasks, and whether pharmacological intervention is possible months after the injury. A single closed head injury was delivered on the somatosensory cortex of male mice at P17 (n=4), matched with sham controls (n=5). INSCOPIX miniscope lenses were implanted in the ipsilateral SC and neurons were transfected to express Gcamp6f 1-month post-injury (MPI). *In vivo* calcium imaging was performed up to 9 MPI at rest and during behavioural paradigms (novel object recognition, elevated plus maze, beam walk, rotarod and hot plate tests). At 11 MPI the possibility of a pharmacological intervention was tested by potentiating the endocannabinoid system.
Basal neuronal activity was increased up to 11 MPI after a jmTBI, indicating a long-lasting hyperactivity of the SC. Behaviourally-induced plasticity of SC neurons was blunted in jmTBI mice specifically in paradigms that increase neuronal activity in sham mice, and this was associated with poor performance in the tasks. We next tested the hypothesis that jmTBIs alter cognitive flexibility by increasing basal neuronal activity, preventing further increase in neuronal activity in response to behaviourally-relevant stimulations. At 11 MPI we pharmacologically reduced basal neuronal activity by blocking endocannabinoid degradation (IP injection of JZL^{184}, 18 mg/kg). This rescued the neuronal short-term potentiation induced by the entry into the open arms of the elevated plus maze and normalized the behaviour.
This work indicates for the first time *in vivo* in a pre-clinical model of jmTBI that neuronal activity is durably altered following a single early life concussion and might play a role in the alteration of neuronal plasticity and associated behaviours. Furthermore, our work suggests that

pharmacological intervention is possible months after the injury, which represents a time-window compatible with clinical treatment. Ultimately, this work will identify the underlying mechanisms involved and might help to identify targets for treatment development.

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Poster

626. Traumatic Injury

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 626.18

Topic: C.10. Brain Injury and Trauma

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Title: Repetitive TBI silences memory through engram cell synaptic changes

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Abstract: Traumatic brain injury is the most common neurological disorder and 80% consist of mild traumatic brain injury (mTBI). Repeat mTBIs (rmTBI) increases the severity and persistence of cognitive symptoms. A high frequency head impact (HFHI) mouse model to replicate rmTBI in sports related contexts was recently developed by the Burns lab. HFHI mice display decreased learning and changes in transcriptomic profiles related to synaptic signaling. These changes were accompanied by decreased plasticity and synaptic changes in CA1 pyramidal neurons. This would suggest that synaptic modifications underlie the anterograde cognitive symptoms following rmTBI. It is still unknown how rmTBI directly effects an already established memory. Engrams are defined as the lasting physical or chemical changes in neurons and are the neural substrate underlying episodic memory. Previous studies have shown that activating this population of neurons can recover amnesia. In this study, we use advances in engram cell labeling to interrogate the mechanisms behind cognitive dysfunction in the repeat head impact brain and whether they are treatable using functional therapies. Using a contextual fear conditioning model, we found that the HFHI model results in a retrograde amnesia phenotype but does not result in loss of memory bearing engram cells. Sham animals displayed an engram cell specific increase in spine volume, AMPA/NMDA ratio, and AMPA decay tau that was abolished by HFHI. Upon optogenetic reactivation of the dentate gyrus engram, the

freezing response was recovered in HFHI, suggesting that memories are silenced, not lost in the repeat head impact brain. These data provide mechanistic insights into retrograde amnesia in the repeat head impact brain and warrant a further investigation into functional treatments for cognitive recovery.

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Poster

626. Traumatic Injury

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UCI Summer Undergraduate Research Program (SURP) fellowships

Title: Brain-wide reconstruction of inhibitory circuits after traumatic brain injury

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Abstract: Despite the fundamental importance of understanding the brain's wiring diagram, our knowledge of how neuronal connectivity is rewired by traumatic brain injury remains remarkably incomplete. Here we used cellular resolution whole-brain imaging to generate brain-wide maps of the input to specific subtypes of inhibitory neurons in a mouse model of traumatic brain injury. In both hippocampus (which was directly injured) and prefrontal cortex (which was not injured), we found that somatostatin interneurons are converted into hyperconnected hubs, with rich local network connections but diminished long-range inputs. The loss of long-range input did not correlate with cell loss in distant brain regions. Interneurons transplanted into the injury site received orthotopic local and long-range input, suggesting the machinery for establishing distant connections remains intact even after a severe injury. Our results uncover a potential strategy to sustain and optimize inhibition after traumatic brain injury that involves spatial reorganization of the direct inputs to inhibitory neurons across the brain.

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Poster

626. Traumatic Injury

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Program #/Poster #: 626.20

Title: WITHDRAWN

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD SC180122
VA BX:004868

Title: Treatment with the 5-hydroxytryptamine 1F receptor agonist lasmiditan post-SCI upregulates SIRT3 deacetylation activity to improve recovery

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Abstract: Spinal cord injury (SCI) decreases oxygen delivery throughout the spinal cord, resulting in a cascade ofsecondary injuries, including mitochondrial dysfunction. Treatment with the FDA-approved 5-hydroxytryptamine 1F (5-HT1F) receptor agonist lasmiditan, induces mitochondrial biogenesis (MB) in thespinal cord and improves locomotor function up to 15 days post-injury (DPI). However at this point, thetherapeutic effect of lasmiditan on locomotor recovery begins to plateau. The mechanism of this plateauremains elusive. Sirtuins (SIRTs) are NAD+-dependent signaling proteins that play vast roles within theCNS. Specifically, mitochondrial SIRT3 is involved in the deacetylation and activation of mitochondrialproteins. Aberrant lysine acetylation of mitochondrial proteins plays a regulatory role in mitochondrialdysfunction of neurodegenerative diseases, indicating that decreased SIRT3 activity can impairmitochondrial function. PGC-1α is considered the “master regulator” of MB. SIRT3 is downstream of PGC-1α activation, suggesting a link between MB induction and SIRT3 function. To investigate the role ofSIRT3 on the altered acetylation of mitochondrial proteins in lasmiditan-induced MB and recovery post-SCI, female C57bl/6 mice were subjected to severe thoracic injury (80 kdyn) and then treated daily withvehicle or 0.1 mg/kg lasmiditan (i.p.) beginning 1h post-SCI. We observed SIRT3 downregulation 3 DPIin the injury site of mice. By 7 DPI, lasmiditan-treated mice increased PGC-1α, mitochondrial transcriptionfactor A (TFAM), and SIRT3 at the injury site compared to vehicle-treated and sham groups. Mitochondrialextracts were used to measure SIRT3 deacetylation activity at 3, 7, 14, and 21 DPI. Similar to increases inPGC-1α, TFAM and SIRT3, SIRT3 deacetylation activity was upregulated 1.4-fold in lasmiditan-treatedmice compared to vehicle-treated group at 7 DPI. However, by 21 DPI, SIRT3

deacetylation activity wasdiminished 2-fold in lasmiditan-treated mice compared to sham controls, connecting to the plateau observedin locomotor recovery. We suggest a role for SIRT3 deacetylation activity in regulating aberrant lysineacetylation to promote lasmiditan-induced recovery post-SCI.

Disclosures: K.J. Bachtle: None. **N.E. Scholpa:** None. **R.G. Schnellmann:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 627.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilson Foundation 725788

Title: Early detrusor application of botulinum toxin A reduced fibrotic bladder development reduced bladder distension and improved bladder function after severe thoracic spinal cord injury in rodents.

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Abstract: Eighty percent of spinal cord injured patients develop debilitating urinary tract dysfunction commonly called neurogenic bladder. Neurogenic bladders are associated with increased rates of bladder infection, autonomic dysreflexia, as well as kidney damage. Current first-line treatment for patients with neurogenic bladders consists of oral anticholinergic medication. However, the side effects of peripheral and central anticholinergic drugs can include physical and mental impairment. Furthermore, anticholinergic medications do not protect against the development of bladder hypertrophy. OnabotulinumtoxinA (BoNT-A) is currently approved for neurogenic bladder after other treatment options fail, at which point, permanent bladder hypertrophy has already occurred. Here, we hypothesized that acute bladder chemodenervation with BoNT-A application into the bladder muscle can prevent the onset of bladder wall hypertrophy after spinal cord injury (SCI) thereby improving bladder function and health post-SCI. Adult female Sprague Dawley rats were given a contusion SCI or a laminectomy-only at spinal level T9. The SCI rats were separated into five treatment and timing groups: SCI only, early saline, late saline, early BoNT-A, or late BoNT-A. Early bladder injections were conducted immediately following the SCI, and late bladder injections were performed 4 weeks post injury (wpi). Cystometry at 6-8 wpi revealed that early BoNT-A bladder injections limited post-SCI bladder distension (capacity) over all other SCI groups (40% reduction from SCI-only group). Regardless of timing, BoNT-A treatment improved bladder compliance over the SCI-only group (2.7-fold increase over SCI-only). Interestingly, bladder function, as it pertains to normal micturition profiles, was maintained in all early BoNT-A animals (3/3); whereas no micturitions

were observed in SCI-only animals (0/5) and few were observed in late BoNT-A treated animals (2/6). Preliminary histological analyses showed a 46% reduction in bladder wall thickness in SCI + early BoNT-A bladders compared to SCI + early saline (0.86 ± 0.08 mm vs 1.6 mm ± 0.25) treated rats. In summary, data presented here suggests that early detrusor chemodenervation following SCI improved bladder function and decreased hypertrophy associated with SCI. Future studies will examine optimal dosage of BoNT-A and the effective therapeutic window to limit hypertrophic bladder tissue development and improved bladder function after SCI.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

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Topic: C.11. Spinal Cord Injury and Plasticity

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Ministry of Education, project number LM2018129 Czech-BioImaging

Title: Orally administrated 4-methylumbelliferone promotes anatomical plasticity and functional recovery in the chronic stage of spinal cord injury.

Authors: *K. ŠTEPÁNKOVÁ[1,2], D. VONDRASEK[3,4], D. HADRABA[4], P. JENDELOVA[1], L. MACHOVA URDZIKOVA[1], J. W. FAWCETT[5,1], J. C. F. KWOK[6,1];
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Abstract: Spinal cord injury (SCI) often leads to partial or complete loss of control of locomotor and sensory functions. Chronic phase is characterized by the stabilization of the lesion including the glial scar, accompanied by alterations in neural circuitries. Axon regeneration is mainly blocked by the inhibitory environment consisting of chondroitin sulfate (CS) proteoglycans and myelin debris. We have previously observed that an oral administration of 4-methylumbelliferone (4MU) at 2.4 g/kg/day is able to reduce the inhibitory matrix at the glial scar in acute treatment. Here, we investigated if a lower dose of 1.2 g/kg/day of 4MU is sufficient. The results demonstrated that long-term 4MU treatment at a dose of 1.2 g/kg/day

downregulated hyaluronan (HA) and, glycosaminoglycans (GAGs) synthesis in uninjured animals, reduced glial scar and promoted the sprouting of serotonergic fibres in animals with chronic SCI. However, 4MU at this dose did not lead to functional recovery in chronic SCI suggesting 1.2 g/kg/day is not sufficient to fully suppress upregulated CSs after SCI. This prompted us to test the previous dose at 2.4 g/kg/day in SCI rats. Rats with SCI were fed for 8 weeks starting 6 weeks after SCI. The treatment was combined with daily rehabilitative treadmill training. We observed the significant locomotor improvement in 4MU treated rats with SCI assessed by BBB, ladder rung walking and max speed test when compared to placebo fed controls. To characterise the axons below and above lesion, we used coherent anti-Stokes Raman scattering (CARS) imaging of myelinated axons and second harmonic generation (SHG) imaging of the surrounding collagen fibres without need for axonal labelling or tissue sectioning. For axonal sprouting tracing we used DiD applied post mortem to the tissue. Our results indicate that 2.4 g/kg/rat 4MU reopened a window of plasticity in chronic SCI, allowing rehabilitation to promote functional recovery.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 627.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH/NINDS 5R01 NS111037

Title: Rolipram delivered by PgP nanocarrier via intrathecal injection restores motor function and reduces neuropathic pain in a rat moderate contusion model

Authors: *J. LEE[1], Z. LIAO[1], J. GAO[1], M. KHANG[1], M. R. DETLOFF[2], K. WEBB[1]; [1]Bioengineering, Clemson Univ., Clemson, SC; [2]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Traumatic spinal cord injury (SCI) is a major source of morbidity and mortality worldwide. Permanent motor, sensory, and autonomic dysfunction, as well as chronic pain, respiratory impairment, and loss of bowel or bladder control result from destruction of neuronal cells after SCI. In our published studies, we demonstrated that rolipram delivered by a polymeric nanocarrier, poly (lactide-co-glycolide)-graft-polyethylenimine (Rm-PgP) restored cAMP level and increased neuronal cell survival and reduced inflammatory response in a rat severe compression SCI model. In our related study, we observed that Rm-PgP single and repeat injection via intrathecal catheter reduced lesion size, inflammatory response, apoptosis, and astrogliosis and improved neuronal survival at 1week post-injury (acute phase). In this study, we evaluated the long-term therapeutic efficacy of Rm-PgP single and repeat treatment via

intrathecal injection on secondary injury, functional recovery, and neuropathic pain in a rat moderate contusion SCI model for 6 weeks (chronic phase). Moderate contusion injury was generated at T9-T10 spinal cord of SD male rats (200-250 g) using an impactor (IH-0400, PSI) with a force of 200 kDyne. Rats were divided into 4 groups: 1) sham, 2) untreated SCI (saline, 40 µl), 3) Rm-PgP-S (Rm-PgP (20 µg Rm, 40 µl) single injection), 4) Rm-PgP-R (Rm-PgP (20 µg Rm, 40 µl) repeat injection at 0, 2, and 4 DPI). Rm-PgP or saline was injected using microinjection pump (WPI, Inc) with Hamilton syringe (28 G) at 2 µl/min. Motor functional recovery and neuropathic pain were evaluated using Basso Bettie and Bresnahan (BBB) scoring system and von Frey test, respectively. At 6 weeks post-injury, rats were sacrificed via cardiac perfusion and the spinal cords retrieved for histological analysis. We observed that Rm-PgP single injection group showed slightly higher BBB score compared to Rm-PgP repeat injection group even though they were not significantly different. The BBB scores of Rm-PgP single injection group were not significantly different from sham group at 6 week time point. For neuropathic pain, hindpaw withdrawl thresholds in both injection groups were higher compared to the untreated SCI groups. Histologically, we observed significantly fewer $ED1^+$ cells and more $Arg1^+$ cells, $NeuN^+$ cells, and higher % spared tissue volume in the spinal cords from animals treated with Rm-PgP compared to those from the untreated group. In conclusion, intrathecal injection of Rm-PgP reduced secondary injury, restored motor function, and reduced neuropathic pain in a rat moderate contusion SCI model.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

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Program #/Poster #: 627.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Verticale
Demain Debout Aquitaine

Title: Sexually dimorphic response to depletion of microglia proliferation after spinal cord injury

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Abstract: Microglia, the immune cells of the central nervous system, exert multiple functions in physiological and pathological conditions. Microglia play a key role in sexual differentiation in the developing brain and display significant gender differences in number and functionality. Besides, microglia are also an important determinant of sexually dimorphic responses in the adult injured brain. After spinal cord injury (SCI) in adult mammals, a scar partly formed by microglia surrounds the lesion and constitutes a barrier that prevents spontaneous axonal

regrowth and thus functional recovery. We have shown that a transient pharmacological reduction of microglia proliferation after injury is beneficial for functional recovery after SCI in mice and nonhuman primates [1, 2]. **Objective and rationale:** here we aim to investigate in mice whether modulation of microglia after SCI induces a sexually dimorphic response. **Methods:** The colony stimulating factor-1 receptor (CSF1R) regulates proliferation, differentiation, and survival of microglia. We orally administrated GW2580, a CSF1R inhibitor that inhibits microglia proliferation in male and female mice (12 individuals per group). Animals were treated for 1 week immediately after SCI (lateral hemisection at thoracic level 9). We analyzed treatment outcomes on locomotor function (open field and Catwalk) and spinal cord pathology (histology). Finally, we used cell-specific transcriptomic analysis to uncover GW2580-induced molecular changes in microglia. **Results:** Males display a better motor recovery in response to the treatment. Females display a less GW2580-induced increase in recovery than males. Similarly, GW2580 administration promotes tissue preservation in male only. Finally, GW2580-treatment in male mice induced down-regulation of proliferation-associated transcripts and inflammatory associated genes in microglia that may account for reduced neuroinflammation and improved functional recovery following SCI. **Discussion and** C**onclusions:** thus, microglia display sexually dimorphic responses to treatment in the adult injured spinal cord. To remove "bottlenecks" in the translation of basic research to preclinic, it is of utmost importance to address fundamental questions in sexual dimorphism after SCI.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

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Topic: C.11. Spinal Cord Injury and Plasticity

Title: Lrrk-2 inhibition by pf06447475 modulates neuronal damage and immunity after spinal cord trauma

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Abstract: Spinal cord injury (SCI) is a devastating event followed by neurodegeneration, activation of the inflammatory cascade and immune system. Dysregulated or non-resolving inflammatory processes can affect neuronal homeostasis and drive immune cells stimulation. The leucine-rich-repeat kinase 2 (LRRK2) is a gene associated with the progression of Parkinson's disease, moreover, its kinase activity was found to be upregulated after instigated inflammation of the Central Nervous System (CNS) and immune system response. Here, we aimed to

investigate PF06447475 role, as a biochemical LRRK2 inhibitor, by counteracting pathological consequences of spinal cord trauma. The in vivo model of SCI was induced by extradural compression of the spinal cord at T6-T8 levels, then mice were treated with PF06447475 (2.5 - 5 and 10 mg/kg i.p) 1 and 6 hours after SCI. We found that PF06447475 treatments at the higher doses (5 and 10 mg/kg) showed great abilities to significantly reduce the degree of spinal cord tissue injury, glycogen accumulation, and demyelination of neurons associated with trauma. In addition, cytokines expression levels include interleukins (IL-1, IL-6, IL-10 and 12), interferon-γ (IFN- γ) and tumor necrosis factor- α (TNF-α) secreted and released by immune cells after trauma were decreased by LRRK-2 inhibitor treatments. Moreover, the accumulation of CD4+ and CD8+ cells throughout the spinal cord lesion site of SCI mice as well CD45+ and CD68+ cells was reduced by PF06447475 administration at the higher dose of 10 mg/kg. Our results suggest that the correlations between LRRK2, neurodegeneration and immunity exist and that LRRK2

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

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Topic: C.11. Spinal Cord Injury and Plasticity

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Title: Synaptic preservation and sciatic nerve regeneration by dimethyl fumarate after spinal cord root avulsion and repair with fibrin sealant

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Abstract: Spinal cord injury causes critical loss of motor and sensory function. In particular, avulsion of spinal roots is a recurrent outcome of high-energy trauma. Experimentally, ventral root avulsion (VRA) can be obtained in rats after an abrupt separation of the motor roots from the surface of the spinal cord, resulting in paralysis of the ipsilateral limb and morphological changes including motoneuron degeneration and local circuitry rearrangements. It is believed that pharmacological treatment associated with root reimplantation can overcome the degenerative effects of VRA, enhancing regeneration and plasticity of the central and peripheral

nervous system (CNS and PNS). Therefore, our goal was to evaluate if dimethyl fumarate (DMF), an FDA-approved drug, when associated with fibrin sealant (FS), a biopolymer used for tissue repair, could improve recovery of motor function after repair of VRA. Thus, adult female Lewis rats were subjected to unilateral VRA of L4-L6 roots followed by FS reimplantation and DMF treatment (15 mg/Kg; daily; gavage) for 4 weeks, being the survival time post-surgery 12 weeks. For that, the following experimental groups were set: VRA+Vehicle, VRA+DMF, VRA+FS+Vehicle, VRA+FS+DMF (n=5/group/technique). All experiments were approved by the committee for ethical use of animals from the University of Campinas (CEUA/UNICAMP: 4873-1/2018). Spinal cord and sciatic nerve samples were evaluated by immunofluorescence and transmission electron microscopy, morphometry of sciatic nerve and the Catwalk system for motor function recovery. Data are presented as the mean ± SEM and compared by one or two-way ANOVA. Mean differences were considered statistically significant when *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$. The combination between FS and DMF was neuroprotective since there was preservation of synapses at the neuropile (****) and nearby the plasma membrane of the alfa motoneurons (****) with restoration of pre-synaptic terminals in comparison to the vehicle-treated counterpart. Also, FS and DMF therapy showed a significant capacity in promoting axonal regeneration observed by the restoration of the 'g' ratio (***) and increased number of myelinated fibers (*). Such parameters were combined with gait recovery, which was translated to improvements in motor coordination (**) and up to 50% of motor recovery (**). Altogether, our results indicate that combining therapies can enhance CNS and PNS plasticity and regeneration due to their cytoprotective effects, showing the potential of FS and DMF for restoring spinal cord functionality.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

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Topic: C.11. Spinal Cord Injury and Plasticity

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Title: NX210c: subcommissural organ-spondin-derived peptide enhances behavioural functional recovery and tissue preservation in cervical traumatic spinal cord injury

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Abstract: Initial physical trauma in spinal cord injury (SCI) is followed by secondary cascades which involve further cell death in the central nervous system and scar formation. Clinically, the highest incidence of traumatic SCI occurs at the cervical level, often with more severe sensorimotor deficits. NX210c is a 12-amino acid peptide derived from conserved thrombospondin type 1 repeat sequences in the subcommissural organ-spondin, which has a unique multifunctional mechanism of action to ameliorate outcomes following neurological injuries. The aim of this study was to evaluate the efficacy of NX210c to promote functional recovery and tissue repair in a cervical traumatic SCI model. Adult female Wistar rats were subjected to a C6/C7 clip compression-contusion injury and treated once daily with intraperitoneal injections of NX210c (8 mg/kg) or its vehicle beginning 4h or 8h post-injury (n=16-17/group). Uninjured sham rats (n=12) received a laminectomy with vehicle treatment beginning at 4h post-injury. Neurobehavioral tests were performed for up to 8 weeks post-injury, and rats were then sacrificed for histological assessments. Early administration of NX210c at 4h increased forelimb grip strength at 3, 4, 7 and 8 weeks post-injury ($p<0.05$) and improved several static and dynamic aspects of locomotion including interlimb coordination, (i.e., regularity index or base of support of the forelimbs; CatWalk). When delaying first administration to 8h post-injury, NX210c promoted weight gain, accelerated bladder control recovery from 14 to 9 days post-injury, and improved trunk balance (inclined plane) as early as one-week post-injury ($p<0.05$). Regardless of the therapeutic window, more SCI rats with weight support were observed following NX210c treatment, however a higher percentage of rats with weight support were observed at the delayed injection timepoint, 94% compared to 75% of corresponding vehicle rats, at 8 weeks post-injury. For skilled reaching (Montoya test), higher accuracy for successful reaching of pellets was observed with delayed injection at weeks 6 and 8 post-injury ($p>0.05$). Using histology (n=6/group) we demonstrate greater white matter preservation and reduced cavity size at the injury epicenter when NX210c treatment is started 8h post-injury compared to vehicle controls ($p<0.05$). NX210c provides a multi-faceted approach that mitigates various aspects of SCI, improving motor function, bladder control, and white matter preservation, with more benefits observed at the later initial injection timepoint. We anticipate that this study will provide a strong proof of concept for the use of NX210c as a treatment for acute cervical SCI patients.

Disclosures: N. Punjani: None. **S. Lemarchant:** A. Employment/Salary (full or part-time):; Head of Preclinical R&D at Axoltis Pharma. **S. Altamentova:** None. **J. Chio:** None. **J. Wang:** None. **Y. Godfrin:** A. Employment/Salary (full or part-time):; CEO at Axoltis Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Shareholder of Axoltis Pharma. **M.G. Fehlings:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research funded by Axoltis Pharma which supplies the NX210c peptide. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NX210c peptide supplied by Axoltis Pharma.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA IK2BX005218
DOD SC180122

Title: DNA methyltransferase inhibition increases locomotor and mitochondrial recovery following SCI in mice

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Dept. of Veterans Affairs/University of Arizona, Tucson, AZ

Abstract: We previously reported that daily treatment with the FDA-approved β_2-adrenergic receptor agonist formoterol (0.3 mg/kg) beginning 8h after spinal cord injury (SCI) induces mitochondrial biogenesis and improves recovery in mice. To investigate the mechanism of this effect, we used TempO-Seq technology to assess gene expression in the injury site of vehicle- and formoterol-treated mice post-SCI. DNA methyltransferase (DNMT) 1 and 3a expression were increased in formoterol-, but not vehicle-treated mice various times post-injury. Concurrently, global DNA methylation was also increased in the injury site of formoterol-treated mice. To determine the role of DNA methylation in recovery post-SCI, injured mice were treated with either formoterol or vehicle as described above, in combination with the DNMT inhibitor decitabine (DAC, 4 mg/kg) on days 7-9, the period in which functional recovery, as measured by BMS score, occurs. Importantly, DNMT activity and DNMT1 protein expression were increased in the injury site of SCI mice 10DPI and this effect was ameliorated with DAC treatment. Interestingly, DAC had no observed effect when combined with formoterol, in that all mice treated with formoterol exhibited increased BMS scores and body weight compared to vehicle-treated mice with or without treatment with DAC, reaching a plateau in functional recovery by 15DPI. In contrast, mice treated with vehicle and DAC did not exhibit a plateau in recovery by 21DPI, demonstrating increased BMS scores compared to those treated with vehicle alone beginning 15DPI and ultimately reaching a BMS score similar to those treated with formoterol. Furthermore, DAC treatment increased the expression of mitochondrial proteins PGC-1α, ATP synthase β and NDUFS1 in vehicle-treated mice 10DPI to levels comparable to that of formoterol-treated mice. Based on these data, the mechanism of formoterol-induced recovery post-SCI is not dependent on DNMT activity. However, DNMT inhibition alone improved functional and mitochondrial recovery, indicating a potential new therapeutic avenue post-SCI.

Disclosures: N.E. Scholpa: None. **R.G. Schnellmann:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NSF Grant DGE-1842165
Center for Regenerative Nanomedicine at Northwestern University
Simpson Querrey Institute for BioNanotechnology

Title: A netrin-1 derived peptide amphiphile influences neurite outgrowth and synaptogenesis through DCC-receptor activation

Authors: *C. SMITH[1,2], Z. ALVAREZ[2,3], R. QIU[4], T. CLEMONS[4,5], F. CHEN[2], J. A. ORTEGA[2,6], H. WELLMAN[4], E. KISKINIS[2,6], S. I. STUPP[1,2,3,4,5];
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Abstract: Following spinal cord injury, the development of the glial scar hinders the physiological and functional recovery of neurons, resulting in chronic paralysis. While exogenous protein delivery can help stimulate axon growth into the lesion site, this approach does not provide a physical scaffold, lacks long term efficacy, and fails to reestablish vital distal connections due to the lack of spatial guidance cues. Peptide amphiphile (PA) molecules, which self-assemble to form high-aspect ratio nanofibers, gel *in situ*, and can be functionalized with bioactive peptide sequences, can provide physical and biochemical cues to damaged neurons. To improve functional outcomes following a spinal cord contusion injury, we developed a novel netrin-1 PA (N1-PA) that presents a previously identified netrin-1 derived cyclic peptide sequence[1]. Using transmission electron microscopy and solution small-angle X-ray scattering, we confirmed that the N1-PA formed high-aspect ratio nanoribbon structures when mixed at 15 and 30 mol% with a non-bioactive PA molecule. Rheological measurements confirmed that these materials formed hydrogel structures with a favorable storage modulus. We next assessed whether the N1-PA could activate DCC-receptor related pathways to influence changes in neurite outgrowth and synaptogenesis. *In vitro* studies using primary mouse E16 cortical neurons showed higher activation of DCC-receptor intracellular pathways when cells were treated with 1uM of N1-PA in solution. 1 DIV after treatment, axons stained with SMI312 revealed that the N1-PA stimulated axon outgrowth to a similar length as recombinant netrin-1 protein (rN1) and significantly longer than neurons with no treatment. To confirm that effects were DCC-receptor specific, we blocked the receptor, which resulted in a significant reduction in both axon length and protein expression in the rN1 and N1-PA conditions. A XonaChip® microfluidics device from Xona Microfluidics was also used to visualize differences in neurite outgrowth over the course of 1 week. Neurites treated with N1-PA and rN1showed a high degree of neurite outgrowth in contrast to a no treatment control. Western blot was used to confirm the upregulation of pre- and post-synaptic markers (synaptophysin and PS95, respectively) in rN1 and N1-PA conditions. These results suggest that implementing the N1-PA as a spinal cord therapeutic could help improve both axon growth and synaptogenesis following injury. To confirm this, N1-PA was tested in a mouse model of mild spinal cord injury to test the effect on neurite outgrowth and functional recovery. **References:** [1]Spilman, P.R., et al, J Alzheimer's Dis, 2016, 52, 223-42.

Disclosures: **C. Smith:** None. **Z. Alvarez:** None. **R. Qiu:** None. **T. Clemons:** None. **F. Chen:** None. **J.A. Ortega:** None. **H. Wellman:** None. **E. Kiskinis:** None. **S.I. Stupp:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
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Title: Recovery of forearm and fine digit function after chronic spinal cord injury by simultaneous blockade of inhibitory matrix CSPG production and the receptor PTPσ.

Authors: *A. MILTON[1], J. KWOK[2], J. MCCLELLAN[1], D. SILVER[3], P. WARREN[4], J. SILVER[1];
[1]Case Western Reserve Univ., Case Western Reserve Univ., Cleveland, OH; [2]Univ. of Leeds, Leeds, United Kingdom; [3]Cleveland Clin., Cleveland, OH; [4]King's Col. London, London, United Kingdom

Abstract: Spinal cord injuries, for which there are limited effective clinical treatments, result in enduring paralysis and hypoesthesia due, in part, to the inhibitory microenvironment that develops and limits regeneration/sprouting, especially during chronic stages. Recently, we discovered that targeted enzymatic modulation of the potently inhibitory chondroitin sulfate proteoglycan (CSPG) component of the extracellular and perineuronal net (PNN) matrix via Chondroitinase ABC (ChABC) can rapidly restore robust respiratory function to the previously paralyzed hemi-diaphragm after remarkably long times post-injury (up to 1.5 years) following a cervical level 2 lateral hemi-transection. Importantly, ChABC treatment at cervical level 4 in this chronic model also elicited rapid, albeit modest, improvements in upper arm function. In the present study, we sought to further optimize and elucidate the capacity for nerve sprouting and/or regeneration to restore gross as well as fine motor control of the forearm and digits at lengthy chronic stages post injury. However, instead of using ChABC, we utilized a novel and more clinically relevant systemic, non-invasive combinatorial treatment strategy designed to both reduce (via oral delivery of 4-methylumbelliferone) and overcome inhibitory CSPGs (via sub-cutaneous delivery of the PTPσ CSPG receptor blocking peptide ISP) simultaneously and spatially extensively. Following a three-month upper cervical spinal hemi-lesion, we show that

the combined treatment has a profound effect on functional recovery of the chronically paralyzed forelimb and paw, specifically during walking as well as precision movements of the digits. Our exciting pre-clinical findings will begin to dramatically enhance our understanding of the basic mechanisms underlying functionally beneficial regenerative events occurring at chronic injury stages for clinically relevant translational benefits.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation Award # 546893

Title: Evaluation of anantin-mediated NPRA inhibition on serum sodium and potassium concentrations in spinal cord injured rats

Authors: *K. M. BEASLEY[1], J. GUMBEL[2], L. ZIPPERER[1], E. SMITH[1], J. E. ARMSTRONG[1], C. HUBSCHER[1];
[1]Anatom. Sci. & Neurobio., Univ. of Louisville Sch. of Med., Louisville, KY; [2]Neurosurg., Univ. of California San Francisco, San Francisco, CA

Abstract: Spinal cord injury (SCI) can lead to severe detriments in multiple body systems, including disturbance of normal urogenital function. Polyuria, or the overproduction of urine, is one of the most common disorders found among individuals with spinal cord injuries. Biomarkers such as atrial natriuretic peptide (ANP) and some of its receptors including the natriuretic peptide receptor A (NPRA) have been implicated in their association with polyuria in spinal cord injured rats. As NPRA functions to balance salt and water homeostasis in the kidneys, its inhibition can be evaluated in part by examining solute balance in serum. Using a moderate T9 contusion model in male adult Wistar rats, chronic delivery of the NPRA antagonist anantin was used alone or in combination with activity-based recovery training (ABRT) with serum taken at pre-SCI, post-injury/pre-ABRT, and end of study (post-ABRT) time points. Although 24-hour void volumes were not significantly different between anantin and vehicle SCI groups, serum concentrations demonstrated a chronic decrease in the level of sodium with a significant acute increase in serum potassium as determined by flame photometry. Blood pressure was also evaluated as the ANP/NPRA system is directly related to the modulation of cardiac hypertrophy seen in cardiovascular failure, and significant reduction in mean arterial pressure (MAP) was observed. The vehicle treated group demonstrated a significantly lower MAP than the anantin treated group post-injury/pre-ABRT. Our results together indicate that the

quantification of serum sodium and potassium levels may prove valuable in examining the mechanisms behind both SCI-induced polyuria and cardiovascular health.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 5R21NS115094

Title: Endogenous Acrolein Scavenging as a Novel Neuroprotective StrategyAfter Spinal Cord Injury

Authors: *S. SUN[1], S. HERR[2], R. STINGEL[4], Z. ZHANG[5], R. SHI[3];
[1]PULSE Integrative Neurosci. Purdue Univ., West Lafayette, IN; [3]Depat. Basic Med. Sci., [2]Perdue Univ., West Lafayette, IN; [4]Ctr. for Paralysis Research/ Weldon Sch. of Biomed. Engin., Purdue Univ., Maple Glen, PA; [5]Purdue Univ., West Lafayette, IN

Abstract: Spinal cord injuries (SCI) result in a slew of secondary biochemical reactions exacerbate the degree and scope of the initial mechanical trauma. Acrolein, a highly toxic aldehyde generated from the oxidative stress-associated lipid peroxidation has emerged as a key mediator in the secondary injury that contributing significantly to the neurological deficits. A key endogenous oxidoreductase, mitochondrial aldehyde dehydrogenase-2 (ALDH2), is known to detoxify acrolein, and therefore play a critical role in acrolein-mediated pathology. Using a novel transgenic (TG) ALDH2*2 deficiency mouse model that recapitulates a clinical genetic condition in 600 million people worldwide, and a recently discovered ALDH2 activator, we planned to assess the acrolein-clearing and neuroprotective role of ALDH2 after SCI through different pathological aspects including inflammation infiltration and axon degeneration.Following a thoracic contusion SCI, the ALDH2*2 TG mice showed an exaggerated ascension of the acrolein level when compared to the wildtype (WT) mice at 2 to 28 days post-injury. In addition, the level of proinflammatory cytokines and macrophage/microglia activation, and axon degeneration, are also significantly higher in the ALDH2 deficiency mice group compared to WT at 1 week post injury. The application of ALDH2 activator rescued the enzymatic activity in the spinal cord, mitigated the post-SCI acrolein elevation in the spinal cord. Accordingly, the lesion area in the spinal cord in the group received ALDH2 activator was significantly decreased compared to the ones without treatment. Behavior tests assessing both locomotor and sensory function revealed a significant better recovery at 4 weeks in both TG and WT treatment groups. This study has further demonstrated the critical role of acrolein in the pathogenesis of SCI, but also the therapeutic value of suppressing acrolein in SCI. Furthermore,

our data also supports a role of ALDH2 as a potential target for anti-acrolein pharmaceutical intervention in neurotrauma diseases.

Disclosures: **S. Sun:** None. **S. Herr:** None. **R. Stingel:** None. **Z. Zhang:** None. **R. Shi:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH/NINDS Grant 5R01 NS111037

Title: Rolipram delivered by PgP nanocarrier via intrathecal injection reduces secondary injury in a rat moderate contusion SCI model

Authors: *Z. LIAO[1], J. GAO[1], M. KHANG[1], K. WEBB[1], M. R. DETLOFF[2], J. LEE[1]; [1]Bioengineering, Clemson Univ., Clemson, SC; [2]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Spinal cord injury (SCI) is a major source of morbidity and mortality throughout the world. One important pathological event after SCI is a significant decrease in intracellular cAMP levels in the injured spinal cord. Phosphodiesterase 4 (PDE4) activation after injury causes degradation of cAMP and this impairs cAMP signaling and affects cell survival. Rolipram (Rm), a PDE4 inhibitor, improves neuronal survival and reduces neuroinflammation after SCI. In our published studies, we demonstrated that rolipram delivered by a polymeric nanocarrier, poly (lactide-co-glycolide)-graft-polyethylenimine (Rm-PgP) restored cAMP level and increased neuronal cell survival and reduced inflammatory response in a rat severe compression SCI model. In this study, we evaluated therapeutic efficacy of single and repeat injection of Rm-PgP via intrathecal catheter on secondary injury at 1 week post-injury (acute phase) in a rat moderate contusion SCI model. Moderate contusion injury was generated at T9-T10 spinal cord of SD rats (200-250 g) using an impactor (IH-0400, PSI, 200 kDyne) and catheter (32 G) was inserted through a hole made in the dura at lumbar level (L4-5). Rats were divided into 4 groups: 1) sham, 2) untreated SCI (saline, 40 µl), 3) Rm-PgP-S (Rm-PgP (20 µg Rm, 40 µl) single injection), 4) Rm-PgP-R (Rm-PgP (20 µg Rm, 40 µl) repeat injection at 0, 2, and 4 DPI). Rm-PgP or saline was injected using microinjection pump (WPI, Inc) with Hamilton syringe (28 G) at 2 µl/min. At 1week post-injury, one set of rats was sacrificed for cAMP level and the other set of rats was sacrificed via cardiac perfusion and the spinal cord retrieved for histological analysis. We observed that both single and repeated Rm-PgP injection restored cAMP level to sham animal level and cAMP levels in both injection groups were significantly higher than that in untreated SCI group. With respect to neuroinflammation, we observed that the number of ED1+ (M1 marker) cells was significantly lower and the number of Arg1+ (M2 marker) cells significantly higher in both Rm-PgP injection groups than that in untreated SCI group. We also

observed that the number of NeuN + cells in both injection group was significantly higher than untreated SCI group, while the % fluorescence intensity from GFAP+ cells in both injection groups was significantly lower than that in untreated SCI group. In addition, the total number of TUNEL+ cells in both injection groups was significantly lower than that in untreated SCI group. In conclusion, local Rm delivery by PgP nanocarrier restored cAMP levels and reduced secondary injury at 1 week post injury in a rat moderate contusion SCI model.

Disclosures: Z. Liao: None. **J. Gao:** None. **M. Khang:** None. **K. Webb:** None. **M.R. Detloff:** None. **J. Lee:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.15

Title: WITHDRAWN

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation
NIH R01HL146477-02
University of MN Division of Physical Therapy

Title: Sex hormone supplementation improves ventilation and the induction of respiratory neuroplasticity male rats with acute spinal cord injury

Authors: *B. DOUGHERTY, J. GRITTNER, R. BAROK;
Univ. of Minnesota - Twin Cities, MINNEAPOLIS, MN

Abstract: Sex hormones act in the central nervous system to regulate respiratory motor output and the expression of respiratory neuroplasticity. We previously demonstrated that 17β-estradiol (E2) is essential for the expression of phrenic long-term facilitation (pLTF) in adult female rats, a form of respiratory neuroplasticity triggered by acute intermittent hypoxia (AIH). Current evidence indicates that E2 is necessary for pLTF expression in male rats as well, through the enzymatic conversion of testosterone to E2 via aromatase. In humans, spinal cord injury (SCI) may reduce circulating sex hormone levels for prolonged periods of time. This injury-induced

loss of sex hormones may temper our ability to induce neuroplasticity and improve function following SCI. Here we tested the hypothesis that supplementation of sex hormones would improve respiratory function and the expression of respiratory neuroplasticity (i.e., pLTF) two weeks following cervical SCI in rats. Adult male Sprague-Dawley rats received a C2-hemisection SCI or sham laminectomy surgery. One-week post-op, a time-release drug pellet was implanted subcutaneously in SCI groups to provide daily hormone supplementation. Groups received either an E2 (β-estradiol 3 benzoate, 10 µg/day), 5α dihydrotestosterone (DHT, 50 µg/day) or a placebo pellet (50 µg/day). DHT is a testosterone metabolite that is not converted to E2, allowing us to parse out the individual effects of testosterone and estrogen on respiratory function and pLTF expression. Two weeks post-op, whole-body plethysmography was used to evaluate respiratory function in awake, freely behaving rats. Placebo treated rats showed an increase in respiratory frequency and reduced tidal volume, typical respiratory responses following a cervical SCI. DHT treatment was similar to placebo ($p = 0.57$) indicating testosterone had little impact on overall breathing function in SCI rats. Rats receiving E2 supplementation, however, showed an increase in tidal volume and slower respiratory frequency closer to the breathing pattern observed in sham control rats in both normoxic conditions ($p = 0.99$) and with respiratory challenge (10.5% O_2, 5% CO_2, $p = 0.25$). Surprisingly, SCI rats receiving *either* E2 or DHT showed a restoration in pLTF magnitude 2 weeks post-SCI ($p < 0.03$ relative to placebo), suggesting that either method of hormonal supplementation may act to restore the capacity for respiratory neuroplasticity at this time point. Collectively, our data indicate that hormone supplementation may promote breathing function and the induction of respiratory neuroplasticity in the early stage of SCI recovery.

Disclosures: B. Dougherty: None. **J. Grittner:** None. **R. Barok:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig Nielsen Foundation 649984

Title: Nimodipine prevents spasticity and enables spontaneous motor recovery after cervical spinal cord injury

Authors: *K. KONKEL[1], C. DOLICK[1], A. KRONER-MILSCH[1], O. KIEHN[3], C. BELLARDITA[4], K. SATKUNENDRARAJAH[2];
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Abstract: Independent of the level of the lesion, about 70% of patients with spinal cord injury (SCI) experience spasticity one year after the injury. Spasticity not only results in abnormal motor activity but may worsen residual motor function and hamper spontaneous motor recovery. Treatment with antispastic drugs interferes with recovery of function, so there is an urgent need for antispastic treatment to reduce spasticity and enable motor recovery. Recently, we showed that genetic silencing of the CaV 1.3 calcium channels prevented the development of spasticity in a mouse model of chronic sacral SCI. Furthermore, prolonged delivery of the drug nimodipine, an FDA and EMA-approved blocker of L-type calcium channels, starting in the acute phase of sacral SCI, prevented the development of increased muscle tone and spontaneous spasms. However, sacral transection injuries are not a common clinical occurrence, and the effects of nimodipine are unknown on the recovery of motor function. This project's overall objective is to examine nimodipine's therapeutic efficacy in mitigating spasticity and enabling motor recovery in a clinically relevant bilateral cervical rat SCI (cSCI) model. Immediately following injury, a six-week regimen of daily nimodipine (10mg/kg) or vehicle solution administration was coupled with electrophysiology and detailed behavioral analysis to assess spasticity and locomotor function. Our data indicate that early and prolonged treatment with nimodipine not only reduced the development of spasticity following injury but also resulted in a significant improvement in tissue preservation and motor recovery compared to vehicle control. Our results indicate that nimodipine is a potential pharmacological treatment to prevent the development of spasticity and promote functional recovery in a clinically relevant model of cervical spinal cord injury.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD SC180122
NIH T32 HL 007249

Title: Treatment with formoterol after moderate spinal cord injury improves thermal hyperalgesia outcomes

Authors: *I. L. PETERSON, N. E. SCHOLPA, K. J. BACHTLE, R. G. SCHNELLMANN; Univ. of Arizona, Univ. of Arizona, Tucson, AZ

Abstract: Approximately 300,000 people suffer from spinal cord injury (SCI) in the United States; SCI is caused by an initial physical insult, whether by injury or disease, and results in partial to complete nerve damage and loss. In addition to the immediate impacts of SCI,

secondary impacts include pain, phantom sensation, respiratory and cardiovascular complications, and loss of sensation and function below the injury site; as such, neuropathic pain is a well-documented and debilitating consequence of SCI. Patient surveys show that 80% of SCI patients suffer from chronic pain, with 40% of those patients stating they would trade functional recovery for pain relief. While the FDA-approved -adrenergic receptor agonist, formoterol, improves mitochondrial function and locomotor capability post-SCI in mice, the effect of formoterol on pain after injury has yet to be determined. Female C57bl/6j mice were subjected to moderate SCI (60 kdyn) followed by daily treatment with vehicle or formoterol (0.3 mg/kg, i.p) beginning 8h after injury and continuing for 6 weeks. Formoterol treatment improved functional recovery in SCI mice compared to vehicle treatment by 7 DPI (BMS score 3 v 2), reaching a final BMS score of 5 v 4, at 6 weeks. All injured mice experienced a 20% weight loss by 3 DPI. Formoterol-treated mice returned to pre-surgery weight by 21 DPI, while body weight was not restored in vehicle-treated mice. Nociceptive pain was assessed via thermal hyperalgesia using a Hargreaves Apparatus with an IR intensity of 50 and automatic cutoff of 20s. Withdrawal latency was measured prior to injury, then weekly beginning 21 DPI, at which point all injured mice displayed a BMS score of at least ~4 and were able to respond to heat stimulation. Formoterol-treated mice displayed increased withdrawal time compared to vehicle-treated mice at 21, 35 and 42 DPI. Additionally, monocyte chemoattractant protein-1 (MCP-1)—a chemokine that has been associated with nociceptive pain and thermal hyperalgesia—was increased and formoterol reduced MCP-1 in treated mice versus vehicle mice at 24h and 72h. We suggest that formoterol treatment not only improves functional recovery post-SCI, but also reduces thermal pain sensitivity, further supporting formoterol as a potential therapeutic option after SCI.

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Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.19

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 5R01NS117103-02
NIH Grant 5R01AI148076-03

Title: Early nanoparticle intervention preserves motor function following cervical spinal cord injury

Authors: *S. E. HOCEVAR[1,2], Y. WANG[1], C. R. CROWTHER[1], B. C. ROSS[1], L. D. SHEA[1,2]; [1]Dept. of Biomed. Engin., [2]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: In addition to the initial physical trauma, spinal cord injury (SCI) triggers an immediate influx of immune cells that secrete pro-inflammatory cytokines and reactive oxygen species that cause secondary tissue damage. Ablation of myeloid cells, however, leads to worse functional outcomes due to their role in wound healing. Therefore, we aim to reprogram these immune cells to promote a less inflammatory and more pro-regenerative environment. Our lab has previously shown that poly(lactide-co-glycolide) (PLG) nanoparticles (NPs) delivered intravenously within 2 hours post-injury (hpi) reduce immune cell infiltration into the spinal cord and enhance motor function. We sought to investigate the window in time which NP administration can successfully modulate the immune response and promote functional sparing. Glucocorticoid treatment is only effective if given within 8 hours of SCI, suggesting that there may also be a critical period for immunomodulation. We hypothesized that NPs must be administered prior to significant accumulation of monocytes and neutrophils to substantially preserve neural circuits and cells. A mouse C5 lateral hemisection was used and resected tissue was replaced with a microporous, biocompatible PLG scaffold. To study the dynamics of immune cell infiltration and secondary tissue damage, mice received 1 mg NPs or vehicle intravenously every 24 hours for 7 days following injury with the first injection starting at the following times: 2, 4, 12, or 24 hpi. At 7 days post-injury (dpi), NP treatment alters the phenotype of infiltrating immune cells. In NP-treated conditions, an upregulation of M2-associated genes and downregulation of M1-associated genes was observed. Additionally, neuronal markers are upregulated in NP-treated mice compared to controls, suggesting greater neuronal sparing. The 2 hpi group had a larger percentage of innervated neuromuscular junctions in the acromiotrapezius and a higher density of motor endplates than mice receiving vehicle or receiving NPs starting at a later timepoint. The increased sparing of neurons and neural circuits in the 2 hpi NP group corresponds with increased motor function, as measured by the number of correct placements and mistakes on a horizontal ladder beam test at 7 dpi. Motor performance for the 2 hpi group stayed consistent through 84 dpi, but motor performance for the other groups continued to improve up to 28 dpi before plateauing. Collectively, these results suggest that early intervention with NPs can modulate the inflammatory response and preserve motor function and circuits following SCI.

Disclosures: **S.E. Hocevar:** None. **Y. Wang:** None. **C.R. Crowther:** None. **B.C. Ross:** None. **L.D. Shea:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.20

Topic: C.11. Spinal Cord Injury and Plasticity

Support: I. Heermann Anesthesia Foundation
the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D), Merit Review Award # 1 I01 RX003986-01A1

the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D), Merit Review Award # 1 I01 RX003123-01A1

Title: Oxygen therapeutic for acute cervical spinal cord injury in a rodent model

Authors: *J. HOU[1,2], D. PLANT[1], S. TSUDA[2,1], B. W. RENGERT[1], A. M. HEATH[1], J. ZHU[2], F. J. THOMPSON[1,3], C. S. GARVAN[2], B. SPIESS[2], P. BOSE[1,2,4];
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Abstract: Spinal cord injury (SCI) is a devastating injury of the Central Nervous System (CNS) that can result in a broad range of life-long locomotor and spasticity disabilities. It significantly and profoundly impacts quality of life. Acute SCI creates a spectrum of tissue destruction. Damage results from both primary injury caused by immediate neural cell death, and secondary injury resulting from ischemic and inflammatory cascades that contributes to lesion expansion and worsening neurological deficit. This "ischemic penumbra" is an obvious target for therapeutic interventions because rapid reduction of ischemia can show substantial benefit for neuron salvage and motor function. It is urgent to address this issue and develop effective therapies that have excellent potential for translation. Therefore, in this study, we evaluated the safety and efficacy of a novel patented IV emulsion of perfluorocarbon (PFC) nanoparticles, $NanO_2$ (NuVox Tucson, AZ), to limit the ischemic penumbra and improve motor disabilities after cervical SCI (CSCI). Moderate $C_{6/7}$ contusion injuries (200 kdynes, Infinite Horizon Impactor) were produced in anesthetized adult rats. Randomized $NanO_2$ (0.6 ml/kg; started 30 minutes after injury and 90 mins apart for a total of 3 doses) or normal saline (NS) was administered via lateral tail vein through a PE-50 catheter. Animals were kept in an incubator with 50% O_2 breathing during the therapy and recovery. *In vivo* Fast spin echo (FSE) MRI was conducted to examine injury lesion size and edema at post-injury (pi) day 1, week 1, week 2, week 4, and week 8 using a 7T MRI System. Locomotor disabilities and therapy-induced improvements were assessed using 3-D kinematic (Vicon Motion Systems) and footprints (CatWalk, Noldus) analyses of gait at pre-injury, pi week 2, 4, and 8. Forelimb grip strength was also measured at pre-injury, pi week 2, 4, and 8. Lower limb spasticity (velocity-dependent ankle torques and time-locked triceps surae EMGs) and H-reflex rate-depression were measured at pi week 4 and 8 using methodology that we developed and reported previously. Our data to date showed the $NanO_2$ treated animals exhibited a) robust improvements in motor functions (spasticity and gait), b) smaller injury volume (FSE T2W MRI), and c) improvements in grip strength. Our results indicate that $NanO_2$ therapy in SCI may have prevented ischemia-mediated secondary neurological damage and improved motor disabilities.

Disclosures: J. Hou: None. **D. Plant:** None. **S. Tsuda:** None. **B.W. Rengert:** None. **A.M. Heath:** None. **J. Zhu:** None. **F.J. Thompson:** None. **C.S. Garvan:** None. **B. Spiess:** None. **P. Bose:** None.

Poster

627. Promoting Spinal Cord Repair: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 627.21

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Combining trifluoperazine and sodium cromolyn avoid cytotoxic and vasogenic edema and prevents sensorimotor impairments after spinal cord injury in rats

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Abstract: Edema formation is one of the very first events to occur after spinal cord injury (SCI) leading to an increase of the intrathecal pressure and consequently to serious spinal tissue and functional impairments. Current edema treatments are still symptomatic and/or non-specific. Since edema formation mechanisms are mainly described as vasogenic and cytotoxic, it becomes crucial to understand the interplay between these two subtypes. Acting on key targets to inhibit edema formation may reduce secondary damage and related functional impairments. In this study we characterize the edema kinetic after T10 spinal contusion using the Infinite Horizon impactor. We use trifluoperazine (TFP) to block the expression and the functional subcellular localization of aquaporin-4 supposed to be implicated in the cytotoxic edema formation. We also use sodium cromolyn (SC) to deactivate Mast cells degranulation supposed to be implicated in the vasogenic edema formation. The sensorimotor performances were assessed weekly by using the BBB score and the inclined ladder test, then the spinal reflexivity (H-reflex) was evaluated 10 weeks after SCI. The kinetic of the cytokine expression during the edema formation were determined using biochemical assays and the estimation of the tissue loss and the glial scar volumes were estimated using a stereological approach. Our results show a significant reduction of edema after separately TFP and SC treatments as well as after combined treatment compared to control. This reduction is correlated with limited sensorimotor impairments. Such results suggest a potential therapeutic interest to limits impairments after SCI in order to highlight the importance of complementary strategies in edema therapy after SCI.

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Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: University of Colorado AB Nexus Grant
National Institutes of Health National Center of Neuromodulation for Rehabilitation, NIH/NICHD P2CHD086844

Title: The effect of repetitive acute intermittent hypoxia on motor learning in persons with spinal cord injury and able-bodied individuals

Authors: A. T. BOGARD, M. R. HEMMERLE, G. R. FISHER, ***A. Q. TAN**;
Integrative Physiol., Univ. of Colorado, Boulder, CO

Abstract: Individuals living with incomplete spinal cord injury (iSCI) have limited walking mobility. Promisingly, several groups have shown that short, repetitive bouts of low oxygen (acute intermittent hypoxia, AIH) improves clinically meaningful measures of walking speed and endurance. How these performance gains are achieved remains unclear. One possibility is that gains in walking performance may in part be attained through improvements in motor learning. In rodent models, AIH initiates de novo synthesis of BDNF, which is associated with enhancements in ladder walking and is implicated in facilitating motor learning. In able-bodied individuals (AB), decreases in metabolic cost during locomotor adaptation tasks track improvements in motor learning. Thus, the purpose of this study is to examine if AIH improves motor learning and metabolic cost during a locomotor adaptation task. To characterize these effects, we quantified spatial and kinetic parameters of walking asymmetry as well as metabolic cost in AB and iSCI participants during a split-belt treadmill motor adaptation paradigm. Participants were randomized into either an AIH or non-AIH control group. AIH consisted of 5 consecutive days of breathing 15, 90-second bouts of hypoxic air (9% O_2) alternated with 60 seconds of normoxic air (21% O_2). Study participants adapted their walking to unexpected, single belt speed perturbations for 4 different conditions: (1) tied-belt baseline with a 1:1 speed ratio, (2) initial adaptation with a 1:1.5 split-belt speed ratio, (3) tied-belt washout, and (4) motor savings with a 1:1.5 split-belt ratio. We found that AB individuals in both the AIH and non-AIH group adapted the leg on the fast belt to generate greater propulsive and braking forces, corroborating prior studies. Similar to previous observations, we found that AB individuals adapt the leg on the fast belt to take longer strides. Further, we observe a reduction in metabolic cost during the motor savings condition compared to initial adaptation condition in AB individuals. Interestingly, we found that the AIH group exhibited similar decreases in metabolic cost to the non-AIH group with lower metabolic costs across conditions. These preliminary results indicate that AIH-mediated enhancements in motor learning may be partially driven by coordination strategies that are more energetically optimal. Future work will determine the contribution of differential muscle activation to motor learning and metabolic cost following AIH. Tailored rehabilitation interventions that enhance the ability to adapt movements to novel environments is critical to translate clinical gains to everyday mobility.

Disclosures: A.T. Bogard: None. **M.R. Hemmerle:** None. **G.R. Fisher:** None. **A.Q. Tan:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R25HD09770
NIH Grant R15NS082711
NIH Grant R25GM061331
CSULA RSCA Mini Grant 2020

Title: Multimodal locomotor training improves stepping in spinal cord injured rats.

Authors: *O. ZARAGOZA RODRIGUEZ[1], S. GUERRA[1], P. SANDOVAL[1], J. ARAIZA[1], J. ZHOU[1], O. SURYAVANSHI[1], S. FLORES[1], N. HENRIQUEZ[1], L. BONILLA[1], A. G. HOWE[2], Y. WANG[1], R. DELEON[1], M. S. JOSEPH[1];
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Abstract: Exercise as a form of rehabilitative strategy has helped animals improve locomotion following a Spinal Cord Injury (SCI). For example, Bodyweight-Supported Treadmill Training (BWSTT) in SCI animals improves hindlimb stepping. There are some doubts if treadmill-specific training has the capacity to translate to overground walking. The circular Bodyweight-supported Ambulatory Rodent Trainer (cBART) incorporates bodyweight support while allowing the animal to ambulate in a quadrupedal gait overground or on a treadmill. The cBART consists of a rotating arm made from lightweight PVC material and is attached to a freely rotating base. One end of the arm is modified to attach a rodent harness, securing the animal, and the opposite end is fitted with an adjustable counterweight to provide bodyweight support. Moving the counterweight along the arm modifies body support on the rodent attached at the opposite end of the arm allowing for continuous overground movement. In this study, we studied the effect of different training methods and conditions on the recovery of locomotor function in spinally contused rats. Spinally contused rats received BWSTT only (n=8), cBART (n=8) only, or the combined training of BWSTT and cBART (n=8) for 8 weeks. Locomotor performance was examined overground and on a treadmill for locomotor recovery. Preliminary data suggests that forward ankle movement as a measure of initiation of step in the hindlimb, paw placement, plantar paw placement, and hip, knee, and ankle excursion angles as a measure for multiple joint functions improved significantly in the combined BWSTT+ cBART trained group when compared to BWSTT alone. This preliminary result suggests the combined locomotor training is optimal for improved locomotor recovery in spinally contused rats. Additional analysis is being performed to examine spinal cord-specific plasticity in these trained groups and their control groups. These findings suggest multimodal rehabilitation strategies may be beneficial and optimize recovery following a contusion spinal cord injury.

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Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NeuroSpark Seed Funding Program 20130009
Craig H. Neilsen Foundation 733278

Title: Targeted recruitment of upper-limb motoneurons using transcutaneous electrical stimulation of the cervical spinal cord

Authors: *J. OH, M. S. SCHEFFLER, C. A. MARTIN, A. G. STEELE, B. VARGHESE, R. L. MARKLEY, D. G. SAYENKO;
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Abstract: Spinal cord injury (SCI) affects approximately 18,000 individuals every year in the United States, with over half of the cases resulting in upper-limb (UL) impairment. Regaining hand function is of the utmost importance for many individuals with SCI. Non-invasive, transcutaneous spinal cord stimulation (TSS) is a novel electrical neuro-modulation strategy that has the potential to increase the excitability of spinal circuits and facilitate functional recovery after SCI. We hypothesized that the individual UL motor pools would be selectively recruited by using specific stimulation locations during cervical TSS, as revealed by motor threshold intensity, maximum amplitude, and the amount of post-activation depression in neurologically intact subjects (NIS). We then implemented this approach in individuals with SCI to examine how the level and severity of injury affects the response of activated motor pools. Eleven NIS and five SCI participants were recruited in this study. We targeted TSS to the cervical spinal cord delivered via a pair of monophasic pulses of 500 μs duration, with inter-stimuli interval of 30 ms using a multi-electrode array. TSS was delivered over the rostral and caudal as well as midline and lateral aspects of the cervical spine. We demonstrated that TSS delivered over the cervical spinal cord in NIS can preferentially activate proximal and distal muscles along the rostrocaudal axis, as well as ipsilateral UL muscles along the mediolateral axis. While rostral stimulation resulted in activation of all tested muscles, caudal stimulation produced a larger magnitude of response at distal muscles. Midline stimulation resulted in a higher probability of activation at proximal muscles, and stimulation at lateral sites resulted in a higher probability of activation at distal muscles. In SCI, the response of activated motor pools obtained from evoked potentials TSS delivered above and below the lesion showed the different motor pools' recruitment along the rostrocaudal and mediolateral axes compared to NIS. Our findings will provide insight for a future large clinical trial in SCI to investigate to what extent the spinal segment-specific effects obtained in a resting state are relevant during different motor tasks, including rehabilitation of UL function.

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Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Buddhist Tzu Chi Medical Foundation TCMF-EP 110-02

Title: Epidural Electrical Stimulation and Exoskeleton Training for Spinal Cord Injury

Authors: *S.-T. TSAI, Y.-C. CHEN, M.-Y. WU;
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Abstract: AbstractSpinal cord injury (SCI) often leads to disconnection between traversing neuronal pathway. The impairment of neural circuitry and its pathway may leave severe and chronic SCI with both motor disability and poor balance control, which is difficult to improve after traditional rehabilitation. Epidural electrical stimulation (EES) and exoskeleton walking training (EWT) could play crucial roles to enhance further rehabilitation interventions in SCI patients, such as gait, yet few studies have investigated the synergistic effect of these clinical interventions so far. Moreover, the training effects of restoring gait or volitional stepping are sometimes limited or take numerous sessions. Based on our pilot study, we have proposed that improvement of dynamic balance control might be an accompanying effect of EWT, which is a more accomplishable therapeutic goal in short-term period. This study aimed to evaluate the combined effects of EES and EWT on dynamic balance control in SCI patients in a short-term training period. This project proposed a 4-week (12 sessions) EWT intervention in SCI individuals after EES surgery. Two SCI patients were recruited in this study. We assessed the sitting and walking dynamic balance by the displacement of center of pressure (CoP) from the force plate and the trunk accelerations with tri-axial accelerometer. After 4-week EWT intervention, the CoP displacement seemed to increase in sitting reaching forward task, while the trunk sway during the walking task had a decreasing trend. The SCI patients receiving short term intensive EWT program after EES surgery seems to improve some aspects of sitting and walking dynamic balance control. These findings raised interesting potential synergic effects of EES and EWT for SCI patients to gain additional rehabilitation benefits.

Disclosures: **S. Tsai:** None. **Y. Chen:** None. **M. Wu:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DoD award#:W81XWH-18-1-0728

Title: The Utilization Effects of Myoelectric Powered Wearable Orthotics in Improving Upper Extremity Function in Persons with SCI

Authors: *G. ANDROWIS[1], A. ENGLER[1], S. AL-RABADI[1], S. RANA[1], S. KIRSHBLUM[2], G. H. YUE[3];
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Abstract: Upper extremity (UE) weakness and/or paralysis following spinal cord injury (SCI) can lead to a limited capacity to perform activities of daily living (ADL). Such disability significantly reduces an individual's level of independence. SCI is a medically complex and life-disrupting condition. An estimated incidence of 17,700 new traumatic SCI cases are reported each year in the United States [1]. In approximately half of these cases, the injury involves some part of the arm and hand (tetraplegia), representing significant disability and dependence for basic functional activities [2, 3]. Restoration of UE motor function in people with SCI remains a high priority in rehabilitation and the field of assistive technology. This abstract describes two male participants with neurologically and functionally stable chronic incomplete SCI, a 75 and a 31-year-old and AIS D and B, respectively, who underwent 18 sessions (over 6 weeks) of UE rehabilitation using a myoelectric powered wearable orthosis (MPWO). This myoelectric device is aimed to help restore function to weakened or paralyzed UE muscles. We examined the handgrip forces, active range of motion (AROM), response time to initiate a movement, and muscles activations before and after rehabilitation training. The response time to initiate UE movements decreased, and handgrip force and handgrip angles improved after training with the UE-MPWO. This early data suggests that the use of this UE-MPWO device may enhance participants' activities that may lead to improved function. The overall goal of this study was to evaluate the effects of UE-MPWO (MyoPro) in ameliorating UE movement impairments to improve ADL and quality of life in people with iSCI. Further study is ongoing, but these initial findings suggest a promise for neurological change by using such a device.

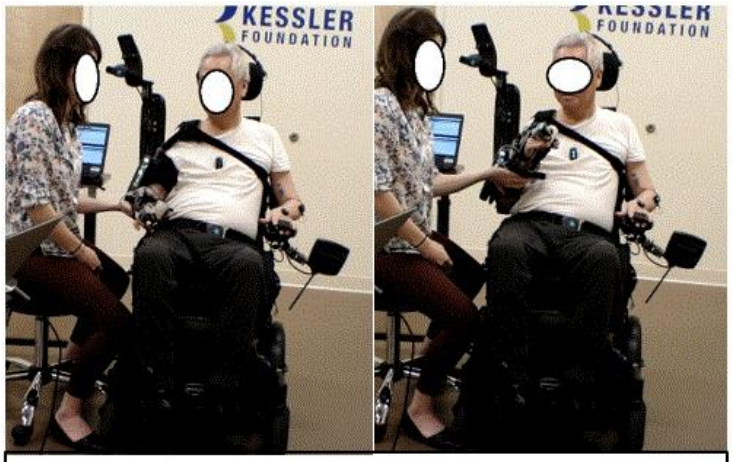

Figure An example of a participant with SCI while utilizing the UE-MPWO (MyoPro) to hold on to an object and bring it closer to his face.

Disclosures: **G. Androwis:** None. **A. Engler:** None. **S. Al-Rabadi:** None. **S. Rana:** None. **S. Kirshblum:** None. **G.H. Yue:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant T32EB000809
University of Arizona Department of Biomedical Engineering startup funds
Core Facilities Pilot Program (CA-CFPP NANO-3310342)
NIH Grant NS112535

Title: Wireless, battery-free and programmable spinal interface for functional electrical stimulation

Authors: A. BURTON[1], ***Z. WANG**[3], S. TRAN[4], J. HANA[2], J. BAKALL[2], D. CLAUSEN[2], J. ANDERSON[2], D. SONG[4], A. BENEDETTO[6], K. SANDEPUDI[4], D. BASRAI[3], E. YANG[4], L. E. MILLER[5], M. C. TRESCH[4], P. GUTRUF[2];
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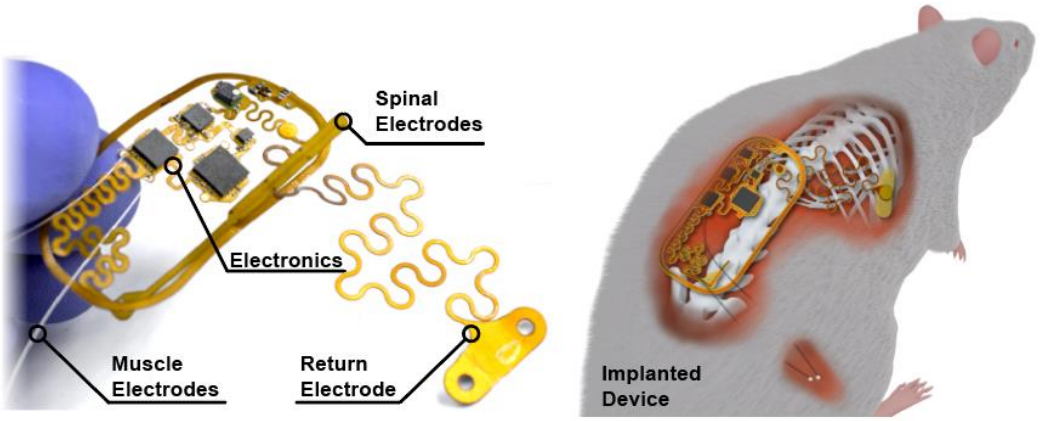
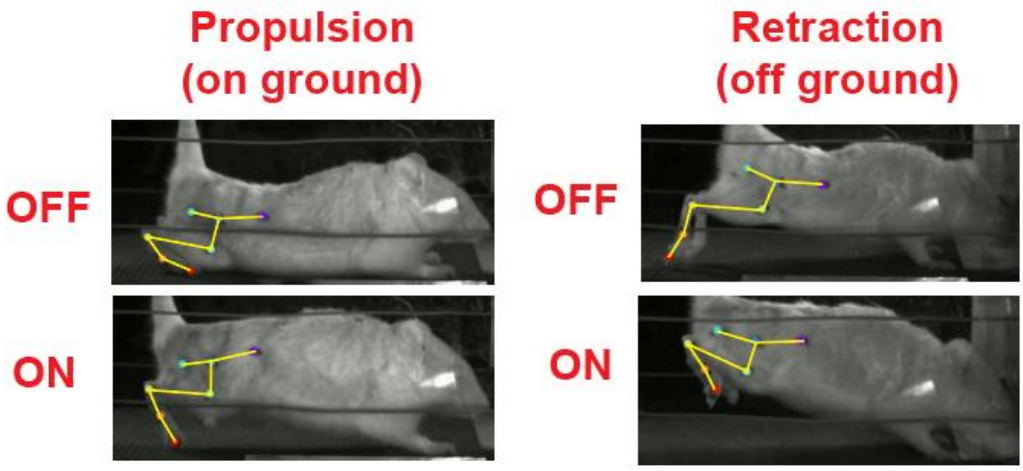
[4]Northwestern Univ., Chicago, IL; [6]Northwestern Univ. Interdepartmental Neurosci., Chicago, IL

Abstract: In this study, we designed a wireless and battery-free platform with efficient power harvesting capabilities to provide high voltage compliance (± 20 V) to deliver a dynamic range of biphasic currents for both spinal and muscle stimulation. The device body is comprised of a thin-film polyimide substrate, populated with off-the-shelf integrated components, and Parylene coated to enable biocompatibility. The thin flexible design and serpentine interconnects allow for deformation and compliance to natural animal movements and behaviors. The high-performance microcontroller and multiplexer provide programmatic control of the wireless platform so that the stimulation intensity could be varied between 0 to 4.7 mA with a resolution as small as 10 µA across up to 8 channels and produce arbitrary sequences of stimulation pulses. We tested the functionality and chronic performance of the device in-vivo evaluating its performance for stimulation of both spinal epidural electrodes and intramuscular electrodes for the production of movement. The animals exhibited well-modulated motor responses and the device could maintain functional performance for several weeks after implantation. We also tested the device in SCI models, showing that the device could be used to produce functional movements such as propulsion in awake behaving animals. The capabilities of this device enable flexible control of a wide range of bioelectrical interfaces, including neurorehabilitation applications.





Disclosures: **A. Burton:** None. **Z. Wang:** None. **S. Tran:** None. **J. Hana:** None. **J. Bakall:** None. **D. Clausen:** None. **J. Anderson:** None. **D. Song:** None. **A. Benedetto:** None. **K. Sandepudi:** None. **D. Basrai:** None. **E. Yang:** None. **L.E. Miller:** None. **M.C. Tresch:** None. **P. Gutruf:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ISRT BBS003

Title: Changes in neural connection of urinary bladder after spinal cord injury and epidural stimulation in rat, a time laps study

Authors: *S. BHATTACHARYYA[1], H. H. CHANG[4], S. CHAKRABARTY[2,3], R. M. ICHIYAMA[5];
[2]Fac. of Biol. Sciences, Sch. of Biomed. Sci., [3]Fac. of Biol. Sci., [1]Univ. of Leeds, Leeds, United Kingdom; [4]Univ. of California, Res. center, Irvine, Lake Forest, CA; [5]Univ. Leeds, Leeds, United Kingdom

Abstract: Changes in neural connection of urinary bladder after spinal cord injury and epidural stimulation in rat, a time laps study Supti Bhattacharyya*[a], Harriet Chang[b], Samit Chakrabarty[a], Ronaldo Ichiyama[a]
a. School of Biomedical Sciences, Faculty of Biological Science, University of Leeds, Leeds, United Kingdomb. Project Scientist, Reeve-Irvine, University of California, Research center, Irvine
*PresenterSpinal cord injury (SCI) causes severe disruption of urinary bladder function. Epidural electrical stimulation (ES) has been shown to improve locomotor function in both animal and human models. There is also some evidence that ES may support recovery of bladder function. The main objective of this time course study was to capture the time window in which an ES intervention could prevent maladaptation of bladder activity after SCI and design a specific paradigm of ES to improve bladder function. Following a severe contusion SCI at T9-T10 level and ES at L2-S1, rats received ES daily starting at 48h or 7 days post injury (dpi). We performed bladder cystometry and electromyography (EMG) of external urethral sphincter (EUS) at 48hrs and 7 days after SCI, and at the end of stimulation periods of 6 weeks. There was a significant ($p<0.0001$) increase in the bladder capacity and maximum bladder pressure (IVPmax) after SCI. We found that bladder capacity and IVPmax were significantly ($p<0.005$) decreased in both ES groups (48h and 7dpi) compared to injured rats without ES. We also observed that ES started 48hours after SCI improved bladder function significantly ($p<0.05$) compared to ES started at 7 dpi. We could see the presence of bursting activity after 48h of SCI which completely abolished

after 7days. With ES there was reappearance of bursting activity in 66.66% and 28.57% in both 48h and 7dpi groups respectively. Abnormal bladder activity was associated with abnormal tonic EUS bursting patterns in all the injured animals. Application of ES after 48hrs has showed significantly better effect than 7dpi group in recovery of bursting pattern of EUS. Our study provides evidence that an early intervention may be necessary to maximize the effects of ES on recovery of bladder function.

Disclosures: S. Bhattacharyya: None. **H.H. Chang:** None. **S. Chakrabarty:** None. **R.M. Ichiyama:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.08

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD Grant WX81XWH-15-2-0045

Title: Therapeutic acute intermittent hypoxia preconditioning enhances enduring training-related gains in overground walking in people with subacute motor-incomplete SCI

Authors: *S. BARTH[1], C. TUTHILL[1,2], W. M. MUTER[1], A. LINK[1], A. LEIFER[1], C. SLOCUM[1,2], R. D. TRUMBOWER[1,2];
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Abstract: Spinal cord plasticity contributes to the recovery of motor function following incomplete spinal cord injury (iSCI). Therapeutic acute intermittent hypoxia (tAIH) has been shown to improve breathing and overground walking ability in persons with chronic (>1 year post injury) motor incomplete SCI. In rats with iSCI, daily sessions of tAIH induce neural plasticity by increasing the expression of plasticity-promoting proteins and strengthening synapses onto motor neurons resulting in profound recovery of walking function lasting weeks. Preclinical studies suggest daily tAIH may be most effective when applied prior to task-specific training and during early-stage recovery when the plasticity-promoting proteins involved are upregulated. Applying tAIH prior to walking practice may enhance skill-based walking practice outcomes in people with subacute injuries (<1 year post injury) who are initially relearning to walk, but this possibility has not been established.
The purpose of this randomized clinical trial was to identify the extent to which tAIH prior to skill-based walking practice may enhance walking recovery in individuals with subacute iSCI. Eleven participants completed 10 sessions (2 weeks) of either 50-min skill-based walking alone (WALK) or tAIH (15, 1.5 min episodes; F_IO_2=0.10±0.01) prior to WALK (tAIH+WALK). Vital signs were monitored during tAIH and WALK to ensure safety. Walking endurance (6-minute

walk test, 6MWT) was assessed at baseline, after treatment on days 5 and 10, as well as 1 week, 1 month, 6 months, and 12 months post-treatment. The assessor was blinded to the treatment group. Eight individuals completed the 6-month follow up (tAIH+WALK = 4, WALK = 4) and 5 participants completed the 12-month follow up (tAIH+WALK = 3, WALK = 2).
All participants improved their 6MWT distance at 1 month (tAIH+WALK: 114.3±63.6m, WALK: 86.4±40.2m) and exceeded the minimal clinically important difference of 36m at 6 and 12 months. However, participants who received tAIH+WALK showed more enduring 6MWT improvements at 6 and 12-month follow-ups as compared to those who received WALK alone. Participants who received tAIH+WALK showed an increase in 6MWT at 6 months compared to baseline (185±78.3m). At the 12-month follow up the greatest increase in 6MWT distance occurred in participants who received tAIH+WALK (255.5±25.3m). Our results offer the first evidence that daily tAIH+WALK enhances overground walking endurance in people with subacute iSCI. These results complement early findings in preclinical models and offer promise for the use of tAIH preconditioning during the early stage of motor recovery after SCI.

Disclosures: S. Barth: None. **C. Tuthill:** None. **W.M. Muter:** None. **A. Link:** None. **A. Leifer:** None. **C. Slocum:** None. **R.D. Trumbower:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
Dana and Christopher Reeve Foundation

Title: Deep brain stimulation of the mesencephalic locomotor region to improve motor function after incomplete spinal cord injury

Authors: *M. I. SCHEUBER[1], A.-S. HOFER[1], C. GUIDOLIN[1], M. E. SCHWAB[1], A. M. SARTORI[2];
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Abstract: Most human spinal cord injuries are anatomically incomplete (incl. many of the functionally complete ASIA A patients), leaving some fibers still connecting the brain with the sublesional spinal cord. Spared descending fibers of the brainstem motor control system can be activated by deep brain stimulation (DBS) of the mesencephalic locomotor region (MLR). The MLR is an evolutionarily highly conserved structure which initiates and controls locomotion in all vertebrates. Acute electrical stimulation experiments in female adult rats with incomplete spinal cord injury conducted in our lab showed that MLR-DBS was able to re-establish a high degree of locomotion five weeks after injury, even in animals with initially very severe

functional deficits and white matter lesions up to 80-95% (Bachmann et al., 2013). Here we analyzed whether MLR-DBS can be used to enable high-intensity locomotor training and long term recovery in rats with large but incomplete spinal cord injuries. First, we show that the effects of MLR-DBS are not present in the first two weeks after the injury but develop over time at 3-4 weeks postop. Importantly, animals retain a high degree of sensory-motor and voluntary control of their movements up to stimulation strengths 60-80% over the motor threshold. Daily sessions of MLR-DBS applied during wading or enriched environment stimulated trainings led to a much higher level of locomotion and training. After 4-6 weeks, animals trained under MLR-DBS showed a higher level of locomotor performance than the rats which trained under non-stimulated conditions or stayed in the home-cage. The MLR does not project to the spinal cord directly; one of its main output targets is the gigantocellular reticular nucleus in the medulla oblongata. Thus, long-term electrical stimulation of spared reticulospinal fibers after incomplete spinal cord injury could enhance their anatomical rearrangement and in this way lead to persistent improvement of motor function. By analyzing the spared, BDA-labelled giganto-spinal fibers we found that their grey matter arborization density 12 wks after injury was lower in the L2 and L5 spinal cord but more normal in terms of layer specificity in the trained as compared to the untrained animals. This pattern could be the result of training/use-dependent refinement and pruning after a compensatory sprouting process, whereby functionally meaningful connections are selectively stabilized. These results could have clinical relevance; a clinical study on MLR-DBS in human paraplegic patients with chronic incomplete spinal cord injury is currently on-going (NCT03053791) with two implanted patients showing encouraging preliminary results.

Disclosures: M.I. Scheuber: None. **A. Hofer:** None. **C. Guidolin:** None. **M.E. Schwab:** None. **A.M. Sartori:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NICHD grant K12HD073945
NINDS grant 5R01 NS111234-4

Title: Ventral horn neural activity influences sensory hyperexcitability after spinal cord injury

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Abstract: Sensory hyperexcitability after spinal cord injury (SCI) is incompletely characterized at the single neuron level. Indeed, characterization of spinal sensory transmission after SCI generally takes a reductionist view, focusing on the activity of specific classes of neurons in the dorsal horn (e.g., wide dynamic range [WDR] and nociceptive specific [NS] neurons). Potential contributions of neurons in other anatomical and/or functional regions of the spinal gray matter are often not considered. As a result, considerable uncertainty continues to surround the mechanisms that drive clinical signs of sensory hyperexcitability such as SCI-related neuropathic pain, spasticity, and spams. Here, we characterized the firing dynamics of spinal interneurons across the dorso-ventral extent of the gray matter in vivo in rats with and without SCI. Experiments were approved by the IACUC of FIU and WUSTL and conducted in adult male Sprague-Dawley rats. Electrophysiological recordings were conducted in 9 uninjured rats and in 8 rats that received a midline T8 contusion 6-8 weeks prior. We simultaneously recorded activity in the dorsal and ventral horns at the L5 dorsal root entry zone. The L5 dermatome was mechanically stimulated by non-painful and painful forces. Outcomes included the number and phenomenological type of recorded neurons during sensory transmission and their temporal features (e.g., firing frequency), and changes in the spatiotemporal profile of neural activity in the dorsal and ventral horns (e.g., multi-unit activity [MUA] spike times). We found that (1) the maximum firing frequency of WDR and NS neurons during nociceptive transmission in injured animals (72.7 Hz and 41.6 Hz, respectively) was higher than in uninjured animals (60.3 Hz and 21.9 Hz, respectively), regardless of behavioral signs of neuropathic pain; (2) the MUA of 8/9 uninjured animals remained stable and showed no evidence of wind-up, whereas injured animals exhibited highly heterogeneous patterns of MUA both during periods of sensory transmission and during periods of spontaneous activity (SA) (e.g., 5/8 animals showed baseline firing rate oscillations, and 4/8 showed periods of spontaneous bursting during sensory transmission); and (3) MUA-derived evidence of windup continued during interleaved epochs of SA, evinced by continual increases in spontaneous discharge rate, spontaneous recruitment of previously silent neurons, or both. Perhaps most surprisingly, these signs of sensory hyperexcitability were driven by neurons in the ventral horn. Our results suggest that neurons in the ventral horn may further enhance pathological sensory transmission after SCI.

Disclosures: **M.F. Bandres:** None. **J.L. Gomes:** None. **J.G. McPherson:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 NS119587-01A1
Wings for Life Foundation (227)

Title: Novel non-invasive approach for standing after spinal cord injury: Combining transcutaneous spinal stimulation, functional electrical stimulation, and robotics

Authors: *D. G. SAYENKO[1], A. G. STEELE[1], J. OH[1], C. A. MARTIN[1], M. S. SCHEFFLER[1], K. MASANI[2];
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Abstract: Standing therapy has various benefits for individuals with spinal cord injury (SCI). Non-invasive techniques, such as transcutaneous spinal stimulation (TSS) and functional electrical stimulation (FES), are preferred for therapeutic purposes. Knowledge on the potential of combining the two modalities for individuals with SCI to enable standing is lacking. We hypothesized that the combination of TSS and FES will result in robust self-assisted and full-body, weight-bearing standing in individuals with chronic SCI. First, we demonstrated that TSS+FES results in higher forces in both knee extensors (KE) and plantarflexors (PF), compared to TSS or FES alone. Our results indicate that force generated by TSS is further augmented and prolonged by the following FES delivered at approximately the midpoint to peak force production, leading to larger force generation. There was a dependency on the timing between the applications of TSS and FES, where we found that utilizing 16.5 to 33 ms delays between FES and TSS yields the most robust response. We also mapped leg muscle recruitment for each stimulation location and generated muscle force. This will be used to optimize stimulation strategies for self-assisted standing even after complete SCI. Second, we developed the Instrumented apparatus for standing (IAS) which incorporates two 3-axis force sensors in the hand-rail support to measure forces exerted by the user's arms on the frame. Lastly, we have developed the Knee-Assist component that attaches to the IAS. The Knee-Assist allows an individual with SCI to perform self-assisted standing by supporting the person's knees with a dynamic force in an assist-as-needed manner. The IAS with the Knee-Assist will quantify the effect of TSS+FES during standing after SCI. Our next objective is to compare self-supported standing performance across TSS, FES, and TSS+FES in a population with SCI. We hypothesize that TSS+FES will maximize the functional gain to promote robust lower body extension revealed by quantitative measurement using IAS.

Disclosures: D.G. Sayenko: None. **A.G. Steele:** None. **J. Oh:** None. **C.A. Martin:** None. **M.S. Scheffler:** None. **K. Masani:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NS104194
Craig Nielson Foundation
Philadelphia Foundation Brody Fund

Title: Impacts of epidural spatial stimulation extent on recovery of function in combined bionic and biological SCI therapies

Authors: *A. P. BORISYUK, K. J. DOUGHERTY, S. F. GISZTER;
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Abstract: Spinal cord injury (SCI) is a debilitating condition without a cure. Our lab has previously demonstrated enhanced locomotor outcomes in rats with complete thoracic 9/10 SCI after combining viral BDNF treatment and pelvis-based robot training. Epidural stimulation (ES) treatment is a promising strategy for treating SCI, but the optimal treatment parameters remain an area of active research. Combining ES with viral BDNF and robot rehabilitation further enhances locomotor outcomes in rodents. Relative to the viral BDNF effects, our prior work has demonstrated a potential critical period during the initial two weeks of training, where ES likely attenuates some spasticity development as side effects of the viral BDNF treatment on motor function. These cumulative spasticity side effects can result in the eventual collapse of gained motor function in ~40% of rats. Over a period of 6 weeks this collapse was completely prevented when broad current spread ES centered at lumbar (L2) and sacral (S1) spinal segments was combined with viral BDNF and robot training. We hypothesized that the treatment of viral BDNF combined with localized ES and robot training would selectively target the central pattern generators at L2 and S1, resulting in improvement in weight-supported stepping throughout therapy. The current study tests rehab combining viral BDNF, robot training and broad versus local ES in rat with complete SCI. We will measure the changes in motor modularity and compare the improvement in functional outcomes by treatment condition. Our data so far show that local ES does not prevent collapse after 6 or 12 weeks in BDNF treated rats if used alone. Unlike local current delivery at L2 and S1, the broad current spread ES did mitigate spastic collapse. While our best combination treatment utilized broad ES, we next aim to improve the therapy with the combined use of an intense but more local ES in parallel to the broad ES current delivery, thus most intensely stimulating only segments promoting central pattern generators for locomotion and extensor support.

Disclosures: A.P. Borisyuk: None. **K.J. Dougherty:** None. **S.F. Giszter:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: WFL-US-10/21-250
Veterans Affairs I01RX002264-01A2
Gordan Masfield Consortium for Spinal Cord Injury 5I50RX001706-06

Title: Rehabilitation Combined with Neural Progenitor Cell Grafts Enables Functional Recovery in Chronic Spinal Cord Injury

Authors: *C. FRERIA, P. LU, M. H. TUSZYNSKI;
Univ. of California San Diego, San Diego, CA

Abstract: Rehabilitation Combined with Neural Progenitor Cell Grafts Enables Functional Recovery in Chronic Spinal Cord Injury
Camila Marques de Freria[1]; Paul Lu[1]; Mark Tuszynski [1]1.Department of Neuroscience, University of San Diego, San Diego, United States.
Within the clinical arena, rehabilitative training is the most widely applicable therapeutic intervention after SCI that shows beneficial outcomes in individuals that have some retained neurological function below the level of injury. Most of the spontaneous recovery along the AIS scale occurs within the first 3 months after SCI, but a small amount of recovery can still be observed at 18 months or occasionally longer. Here we aim to combine rehabilitation with stem cell therapy. Previously we reported that neural progenitor cell (NPC) grafts form neural relays across sites of spinal cord injury (SCI) and support functional recovery. We now examine whether NPC grafts after *chronic* delays of one-month post-injury in a severe contusion model of SCI support recovery, and whether intensive rehabilitation further enhances functional outcomes. Notably, the combination of rehabilitation with NPC grafts significantly enhanced host corticospinal regeneration into grafts compared to animals with no rehabilitation (grafts alone) and further supported functional improvements compared to controls. Collectively, these findings identify a critical and synergistic role for neural plasticity driven by rehabilitation in supporting functional recovery after stem cell therapy.We next aim to identify the rehabilitative transcriptome of corticospinal neurons in efforts to understand molecular mechanisms associated with rehabilitation-driven functional improvements.To date, the molecular characterization of rehabilitation has not been identified. Using modern tools of neuroscience, we aim to parcel out the effects of rehabilitation on motor systems, and to further utilize these findings towards improved therapies for SCI.

Disclosures: C. Freria: None. **P. Lu:** None. **M.H. Tuszynski:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.14

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Hospital outcomes following fusion for spine curvature in pediatric spinal cord injury: analysis using the national inpatient sample

Authors: *B. UGILIWENEZA[1], G. SINGH[2], M. SHARMA[1], D. WANG[1], M. BOAKYE[1], A. L. BEHRMAN[1];
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Abstract: Scoliosis develops in nearly all cases of pediatric on-set spinal cord injury (SCI). The incidence of kyphosis and lordosis have been documented to be 64% and 20% respectively in this population. Sixty eight percent of these children who develop spine curvature require spinal fusion. The aim of this project was to evaluate hospital outcomes and charges for hospitalizations of surgical corrections of spinal deformity in children with SCI. We used data from National Inpatient Sample (1998-2019) to identify hospitalization for children (0-17) with SCI undergoing fusion for spinal curvature using ICD-9 and ICD-10 codes. Demographic, comorbidities, socioeconomic, and hospital characteristics were noted. Outcomes evaluated were critical care intervention (CCI), hospital length of stay and charges, development of medical complications, and discharge disposition. Generalized linear regression models were used for statistical analysis. A cohort of 633 children, average age 14 (SD=6), 49% females, 68% White, 10% Black, and 14% Hispanic were identified. The majority had no comorbidities (78%), and Medicaid constituted 34% of cases. Most surgeries took place in large (69%) and urban teaching (93%) hospitals. CCI was received in 22% of the cases, and 43% developed medical complications during the surgery hospitalization. Mortality was low (<2%) and 44% of children were discharged to home healthcare or to a medical facility. Median length of stay was 8 days (interquartile range: 5-15) and median charges were $218,604 (interquartile range: $128,826-$336,274). Black children were less likely to receive CCI, but more likely to stay longer and have higher total charges than White children. Hispanic children had comparable CCI with shorter stay, but higher charges than White children. Children on Medicaid were less likely to receive CCI but had longer stay and higher charges. Younger age was associated with more likelihood to be discharged to more health care. Cases treated in teaching hospitals were more likely to receive CCI, had longer stays and higher charges. Fusion for spine curvature in children with SCI results in low mortality but substantial morbidity with weeklong length of stay, hundreds of thousands of dollars in hospital charges, and need for further care after discharge. Effort should be put into rehabilitation, treatment, and management preventive interventions.

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Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS107807

Title: A system for semi-automated administration and digital analysis of a pellet retrieval task in mice

Authors: *L. FRIEDRICH[1], M. BLACKMORE[2], Z. WANG[3], N. CHHIKARA[3], N. SAMBERG[3], I. ANTOLAK[3];
[2]Biomed. Sci., [1]Marquette Univ. Dept. of Biol. Sci., Milwaukee, WI; [3]Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: A system for rapid, multi-animal administration and analysis of a pellet retrieval task in mice Logan Friedrich, Zimei Wang, Neil Chhikara, Nick Samberg, Isabel Antolak, Murray BlackmoreRetrieval of food pellets is widely used to assess forelimb function in animal models including mice. The administration and analysis of pellet retrieval can be laborious when performed manually, motivating efforts to create automated systems of delivery and computer-assisted methods of scoring. We have assembled a system that allows a single user to administer a pellet retrieval task to sixteen mice simultaneously, with each animal reaching through a narrow slit to attempt retrieval of twenty to eighty sequentially presented pellets. Each animal is digitally recorded, and the position of the pellet, nose, and paw is tracked continually using DeepLabCuts. In addition, 3D printing allows precise control of size and shape of the pellet tray, enabling systematic testing of how changes in the geometry of presentation impact the success of retrieval. Although the contribution of the corticospinal tract (CST) is often emphasized, various subcortical projections, especially brainstem-spinal, are also known to contribute to elements of targeted reach, grasp, or retrieval. Taking advantage of the throughput afforded by this system we have tested cohorts of animals using various tray configurations, for example placing pellets within depressions or atop pillars to disallow “scooping” solutions, and then assessed reliance on the CST by retesting after unilateral ablation by pyramidotomy. In addition, the semi-automated system facilitates repeated exposure of injured animals to the task with tray geometries of graded difficulty as a form of progressive rehabilitation. Interestingly, some geometries mostly prevent successful retrieval after pyramidotomy, indicating initial CST dependence, but even with the most complex geometries we find that animals eventually regain retrieval ability after graded rehabilitation, indicating learning of CST independent solutions. Ongoing experiments are mining DeepLabCuts limb tracking data for information about potential compensatory motions in injured animals and using cFos immunohistochemistry to identify subcortical brain regions whose activity correlates with task success. These data establish a rapid means to assess forelimb behavior in mice and the first steps in clarifying the neural basis for CST-independent solutions to targeted object retrieval.

Disclosures: L. Friedrich: None. **M. Blackmore:** None. **Z. Wang:** None. **N. Chhikara:** None. **N. Samberg:** None. **I. Antolak:** None.

Poster

628. Spinal Cord Repair with Electrical Stimulation and Training: Animal and Human Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 628.16

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Characterization of Clinical trials for spinal cord injury - Current reports from the ClinicalTrials.gov registry

Authors: *F. MOINUDDIN, M. BYDON, C. ONYEDIMMA;
Mayo Clin., Mayo Clin., Rochester, MN

Abstract: Study Design: Retrospective Database Study
Objectives: An effective treatment modality for spinal cord injury (SCI) does not yet exist despite the field's breadth of trials. The current study's objective was to outline the characteristics of clinical trials targeting SCI and identify potential areas for improvement to guide future trials.
Methods: The Clinicaltrials.gov database was queried for the baseline characteristics and detailed information regarding trial design with SCI from October 1999 to April 2020. To assess the different characteristics, trials were separated into interventional and observational based on their study design. Multivariable logistic regression analyses were performed for interventional trials to identify factors associated with using randomization, blinding, or a data monitoring committee (DMC) in trial design.
Results: Of 1,061 SCI trials identified, 83.13% were interventional and 16.87 % were observational. At the time of this review, 42.1% of trials were listed as completed status for interventional while 48.6% were for observational. Most trials were registered before the year 2008 for both interventional and observational. NIH primarily sponsored only 1.2% of these trials. Factors associated with randomization, blinding and a DMC included intervention type, phase, and intervention model. In this study, later phase trials had lower odds of utilizing a DMC. Parallel group intervention trials had lower odds for randomization.
Conclusions: ClinicalTrials.gov database was a useful tool to characterize the present and completed trials focusing on SCI. Although many trials have missing information, we observed differences in enrollment, primary funding source and endpoint. Special attention should be given to randomization, blinding, and DMC to improve future clinical trial design.

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Poster

629. Spinal Cord

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 629.01

Topic: D.02. Somatosensation – Pain

Support: R01NS112632
T32NS073548

Title: Spinal S1PR1 Agonism Reduces Neuropathic Pain in Multiple Sclerosis

Authors: *S. R. LAMERAND[1], K. L. NGUYEN[2], B. C. SHAW[3], B. K. TAYLOR[2];
[1]Ctr. for Neurosciences, [2]Anesthesiol. and Perioperative Med., Univ. of Pittsburgh, Pittsburgh, PA; [3]Physiol., Univ. of Kentucky, Lexington, KY

Abstract: Sphingosine-1-phosphate receptors (S1PRs) are an emerging target for the treatment of persistent pain. An emerging literature reports that the S1PR agonist / functional antagonist fingolimod, an FDA-approved disease modifying agent for multiple sclerosis (MS), reduces pain-like behaviors in models of inflammatory and neuropathic pain. For example, we reported that systemic administration of fingolimod reduced mechanical allodynia in the experimental autoimmune encephalomyelitis (EAE) model of MS. This reduction in mechanical allodynia was mimicked by the S1PR1 agonist SEW2871 and blocked by the S1PR1 antagonist, W146, indicating an S1PR1-dependent mechanism. However, the anatomical sites of action remains unclear. Peripheral versus central S1PR signaling can elicit opposing effects with the endogenous S1PR ligand, S1P, being generally pronociceptive in the periphery and antinociceptive in the CNS. Intrathecal (i.t.) injection of S1P reduced pain behaviors in the formalin assay while injection in to the DRG produced peripheral sensitization. Preliminary data demonstrates that i.t. injection of fingolimod, the S1PR1 agonist CYM5442, or the S1PR1/R5 agonist BAF-312 each decrease EAE-induced mechanical allodynia. In the present study we tested the hypothesis that spinal S1PR1 agonist actions of fingolimod reverse multiple sclerosis associated pain (MSNP) behavior in EAE. Adult male and female C57BL/6 mice underwent EAE induction, and all mice developed signs of neuropathic pain, i.e. mechanical and cold allodynia, within 14 days of induction that remained present until the conclusion of the study. Mechanical allodynia was tested with the application of von Frey hairs (up/down method) to the plantar surface of the left hindpaw. Following the development of allodynia, mice received an intrathecal injection of either fingolimod (1ug/5ul), SEW2871 (1ug/5ul), the S1PR1 antagonist NIBR0213 (1.5ug/5ul), fingolimod+NIBR0213 (5ul), or vehicle (5ul) followed by assessment of mechanical allodynia from 30-120 minutes post injection. We found that both fingolimod and SEW2871, but not NIBR0213, vehicle or FTY720+NIBR0213 significantly attenuated mechanical allodynia with peak effect at 60 minutes post i.t.-injection. We conclude that the spinal cord engages S1PR1 agonism to reduce mechanical allodynia in the EAE model of neuropathic pain. These results point to spinal S1PR1 as a target for future pharmacotherapy of multiple sclerosis associated neuropathic pain.

Disclosures: S.R. Lamerand: None. **K.L. Nguyen:** None. **B.C. Shaw:** None. **B.K. Taylor:** None.

Poster

629. Spinal Cord

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 629.02

Topic: D.02. Somatosensation – Pain

Support: NINDS Grant R01-NS093990
NCATS Grant UL1TR002649
CCTR Endowment Fund of Virginia Commonwealth University

Title: Sphingosine-1-phosphate receptor type 1 (S1PR1) deletion in selected cell types indicates a predominant functional localization of S1PR1 in astrocytes in the central nervous system

Authors: *A. M. PONDELICK[1], P. MARCELLI[2], O. DOMINGUEZ[2], S. SINGH[2], S. SPIEGEL[2], K. F. HAUSER[1], A. H. LICHTMAN[1], L. J. SIM-SELLEY[1], D. E. SELLEY[1]; [1]Pharmacol. & Toxicology, [2]Biochem. and Mol. Biol., Virginia Commonwealth Univ., Richmond, VA

Abstract: Sphingosine-1-phosphate (S1P) is a bioactive lysolipid that acts as the endogenous ligand for G-protein coupled S1P receptors (S1PR) types 1-5. The S1PR system has been targeted for immune modulation, but research indicates its potential for pain relief. FTY720 (FTY) is a S1PR immunomodulatory prodrug that activates S1PR 1, 3-5 and is FDA approved for multiple sclerosis treatment. Evidence has indicated that S1P and FTY produce antinociception in models of acute and neuropathic pain, where results with S1PR1 selective agonists and antagonists suggest this likely occurs through S1PR1. Here we used conditional gene deletion to determine the cell type(s) mediating G-protein activation in the spinal cord and brain, using agonist-stimulated [^{35}S]GTPγ;S binding. We hypothesized S1PR1 was the predominant S1P-receptor in the central nervous system (CNS), and astrocytes were the predominant cellular localization of S1PR1 in the CNS. Deletion of S1PR1-floxed male mice crossed with cre-recombinase under the control of the nestin-cre (NC) promoter, which deletes S1PR1 from neurons, astrocytes, and oligodendroglia, and deletion of S1PR1 in both sexes with a glial fibrillary acidic protein (GFAP)-cre promotor both showed a profound decrease in G-protein activation stimulated by S1P (~75-85%) or the S1PR1 agonist SEW2871 (SEW) (>95%) in the spinal cord (NC: n=4-5, GFAP: n=6-11). [^{35}S]GTPγ;S autoradiography in brain slices from these mice also result in a profound loss of agonist-stimulated G-protein activation. The loss of S1P- and SEW-stimulated G-protein activation in the GFAP-S1PR1 conditional knock-out (cKO) mice suggest the majority of S1PR activity is mediated by S1PR1 and S1PR1 activity is mainly localized in astrocytes in the CNS. We hypothesized the remaining 15-25% S1P-induced stimulation in our cKO models may be due to S1PR3 activation; however, global deletion of S1PR3 in male mice (n=4) resulted in no significant difference in S1P- or SEW-stimulated G-protein activation in lumbar spinal cord between the S1PR3 KO and wild-type mice. Furthermore, 14-day FTY treatment in male and female GFAP-S1PR1 cKO mice (3 mg/kg; n=4-8) showed a significant decrease in S1P- and SEW-stimulated G-protein activation in the spinal cord to the same level as the cKO mice, suggesting FTY-induced desensitization of S1PR1 in the lumbar spinal cord is likely due to actions at S1PR1 on astrocytes. These results reveal functional S1PR1 localization in the CNS and support evidence for a role for S1PR1 in astrocytes in FTY720-responsive pain models.

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Poster

629. Spinal Cord

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 629.03

Topic: D.01. Somatosensation

Title: A single cell and spatial atlas of the spinal cord identifies conserved cell types with divergent transcriptional programs across species

Authors: *J. MAKSYMETZ[1], M. DOURADO[1], M. JUNG[2], H. HUANG[3], O. FOREMAN[4], T. BIANCALANI[3], J. S. KAMINKER[2], D. H. HACKOS[1];
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Abstract: The dorsal horn of the spinal cord integrates and processes diverse sensory information while its dysfunction contributes to chronic pain. Within the dorsal horn, an intricate network of excitatory and inhibitory neurons supported by a myriad of glia integrate sensory input and ultimately relay this information to higher-order brain structures. While recent work has characterized the cellular diversity of the mouse spinal cord, there remains a critical gap in our understanding of the various cell types in higher-order species. To address this, we performed single nucleus RNA sequencing (snRNAseq) of lumbar spinal cord tissue from the mouse, rat, cynomolgus macaque, and human. Cross-species integration and analysis of over 200,000 single nuclei identify that broad cell types are conserved at a transcriptional level across species. Specific neuronal subtypes described in the mouse are also conserved in the rat, macaque, and human, allowing us to define a cross-species transcriptional classification of spinal cord neurons. Our integrative analysis further reveals species-specific differences in cell type abundance and transcriptional programs such as differential expression of ion channels and neuropeptides relevant to spinal cord function and human disease. Given the critical role distinct spinal laminae play in sensory processing, we performed spatial transcriptomics on human spinal cord sections using 10X Visium to assess if the spatial localization of neurons is also conserved across species. Using the deep-learning method Tangram, we aligned our human snRNAseq and spatial datasets to determine the spatial distribution of neuronal and non-neuronal subtypes in the human spinal cord and compare this to existing mouse spinal cord spatial atlases. We also describe sex-specific differential gene expression, differences in receptor-ligand interactions, and differential expression of genes implicated in chronic pain and neurodegenerative diseases across species. Altogether, these data provide a comprehensive single cell and spatial atlas of the spinal cord across preclinical species and humans to aid in the functional characterization and discovery of novel therapeutic strategies for the treatment of pain and neurodegenerative disorders.

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Kaminker: A. Employment/Salary (full or part-time):; Genentech, Inc. **D.H. Hackos:** A. Employment/Salary (full or part-time):; Genentech, Inc.

Poster

629. Spinal Cord

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Program #/Poster #: 629.04

Topic: D.01. Somatosensation

Support: CIHR PJT-162225
CIHR PJT-178275
CIHR PJT-153053

Title: Genetic identification of a spinothalamic pathway for somatosensory integration during locomotion

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Abstract: Somatosensory inputs are relayed from the spinal cord to various supraspinal targets, including the thalamus, but the functional organization of such pathways remains obscure. While the lateral spinothalamic tract (STT) has been primarily associated with the relay of noxious signals to the thalamus, the ventral component of the STT is proposed to relay crude touch and joint movements during locomotion in mammals. The cardinal transcriptional programs in the embryonic spinal cord provide insights into the molecular identity of these two STT components. Our previous work shows that lateral STT neurons derived from the lineage expressing Phox2a and Lmx1b transcription factors, contribute to the anterolateral tract involved in the relay of noxious and thermal inputs. Here, we present evidence that the neurons of the lumbar ventral STT arise from Sim1-expressing V3 neurons. This developmental divergence is mirrored by differential thalamic innervation: dI5-STT neurons primarily innervate the medial and the posterior limiting nuclei, while the V3-STT neurons predominantly innervate the ventral posterolateral (VPL) nucleus and the posterior complex. To study the role of V3-STT neurons, we used mouse intersectional genetics to ablate them and their post-synaptic VPL thalamic neurons. V3-STT neuron or VPL ablation did not alter reflexive or conscious responses to noxious stimuli. In contrast, such manipulations lead to deficient behaviors associated with the detection of innocuous touch and coordinated locomotion. Recent functional manipulation of dorsal column nuclei inputs to the VPL implicate it as a somatosensory relay site critical for forelimb movement. As past studies suggest that V3-STT neurons are activated by joint movement and muscle stretch, they may comprise a parallel ascending pathway for hindlimb kinesthesia. Thus, the somatotopically organized VPL is emerging as a major hub for the relay of non-noxious somatosensory inputs to the primary somatosensory cortex during locomotion.

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Poster

629. Spinal Cord

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Program #/Poster #: 629.05

Topic: D.01. Somatosensation

Title: Local VNC sensorimotor circuits of leg rubbing in Drosophila

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Abstract: Most limb movements are controlled by sensorimotor circuits, which require coordination of multiple groups of neurons across the nervous system. Drosophila grooming is a highly coordinated behavior between the fly's six legs controlled by complex neural circuits using sensory information to initiate motor responses. The Drosophila nervous system consists of the brain and the ventral nerve cord (VNC). Behavioral evidence suggests the VNC also to external stimuli in grooming by generating motor outputs: decapitated flies can perform different grooming movements when covered by dust. We are interested in how the local VNC sensorimotor circuits can produce diverse and precise cleaning actions.
From our optogenetic activation screen, we found three homologous pairs of command-like neurons, one pair in each segment of the VNC, which can trigger leg rubbing in undusted flies. We call these Leg rubbing Projection Neurons (LegPNs). These are ascending neurons with projections to the brain, but activation still initiates leg rubbing in decapitated flies, suggesting local VNC circuits are sufficient for LegPN-induced leg rubbing. The three pairs of LegPNs each initiate leg rubbing but have anatomical differences based on where they are located in the VNC, suggesting differences in types of leg rubbing upon activation. We performed clonal experiments to test what leg rubbing mechanisms would be performed upon activation of each LegPN. Activation of a single LegPN triggered different leg rubbing movements based on the region of the VNC it is concentrated in. We used light-level microscopy to characterize the shape/morphology of the LegPNs and then located them in the Female Adult Nerve Cord (FANC) electron microscopy dataset. We found LegPNs receive direct sensory input from tactile bristle, wing campaniform, and bitter sensory neurons from the targeted leg. These sensilla are known to be able to trigger grooming. The EM connectome analysis reveals other neurons pre-synaptic to LegPNs whose functions are not yet known. LegPNs have several different pre-motor neurons downstream, including a group we named the LegPLs. LegPLs have three main outputs; motor neurons, sister LegPLs, and the LegPN. Connection onto sister LegPLs may work to

increase level of motor response by initiating parallel pathways. The positive feedback loop created by LegPLs synapsing onto LegPNs can continue the signal. Our next step is to test the behavioral roles of the circuits we mapped using the EM connectome data. Our study shows VNC local circuits are sufficient for leg rubbing movement, including sensation, central information processing, and also generation of a coordinated motor program

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Poster

629. Spinal Cord

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Topic: D.01. Somatosensation

Support: National Funds through FCT—Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04293/2020

Title: Contralateral afferent input to lumbar lamina I neurons as neural substrate for mirror-image pain

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Abstract: Mirror-image pain arises from pathological alterations in the nociceptive processing network that controls functional lateralization of the primary afferent input. Although a number of clinical syndromes related to dysfunction of the lumbar afferent system are associated with the mirror-image pain, its morphophysiological substrate and mechanism of induction remain poorly understood. Therefore, we used ex-vivo spinal cord preparation of young rat to study organization and processing of the contralateral afferent input to the neurons in the major spinal nociceptive projection area lamina I. We show that some decussating branches of thin primary afferents reach the contralateral superficial dorsal horn, where they contact somata and dendrites of spinal neurons. In agreement with this, we have found that, in the lateral part of lamina I, 10% of neurons, including projection neurons, receive direct inputs from contralateral Aδ and C afferents. The overall mono- and/or polysynaptic excitatory input from contralateral afferents was recorded in 27% of neurons. All these neurons also received their ipsilateral afferent input which was considerably stronger than the contralateral one. Our data show that the contralateral Aδ and C fiber input is under inhibitory control. Removal of the afferent-driven presynaptic inhibition and/or disinhibition of the dorsal horn network increased the contralateral excitatory drive to lamina I. Thus, these results show that some lumbar lamina I neurons are wired to the contralateral afferent system whose input, in normal conditions, is under inhibitory gate control.

A pathological disinhibition of the decussating afferent pathways can open a gate controlling contralateral information flow to the nociceptive projection neurons, and thus, contribute to induction of mirror-image pain.

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Poster

629. Spinal Cord

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Program #/Poster #: 629.07

Topic: D.01. Somatosensation

Support: Offerfonden (Denmark), grant #: 19-610-00055

Title: Distribution of spinal PKD2L1-expressing cerebrospinal fluid contacting neurons across rodent, carnivore, and primate

Authors: *X. LIU[1], S. M. NASSERI[1], K. RICH[1], T. H. HOLBEK[1], B. FINSEN[1], H. SCHERBERGER[2], H. HULTBORN[3], Å. F. SVENNINGSEN[1], M. ZHANG[1];
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Abstract: Cerebrospinal fluid contacting neurons (CSF-cNs) are a specific type of neurons located around the ventricles in the brain and the central canal in the spinal cord and have long been suggested to have intrinsic sensory properties in the central nervous system (CNS) of several animal species. The CSF-cNs play an essential role in maintaining homeostasis in CNS by sensing pH and pressure changes in CSF. One of the important channels which is responsible for these functions is polycystic kidney disease 2-like 1 (PKD2L1). Studies investigating the distribution and functions of these neurons in the spinal cord have previously been primarily performed in non-mammalian vertebrates, such as fish. In mammalian vertebrates a large body of studies about these neurons were in mice owing to the availability of transgenic models. Detailed studies about the distribution of CSF-cNs in the CNS in other species are sparse or lacking. In the present study immunohistochemistry was used to determine the distribution of PKD2L1-positive CSF-cNs in the spinal cords of mice, rats, cats, and macaque monkeys. In addition, in-situ hybridization was used to detect PKD2L1 mRNA expression in the rat spinal cord. We found that, although with a slight difference in their distribution in relation to their locations and expressing patterns, PKD2L1-expressing neurons were found in all the segments throughout the entire spinal cord in all these animal species. Generally, mice and rats shared similar expressing patterns, i.e., the cell bodies of PKD2L1-positive neurons were clearly visible around the central canal with some being distributed a bit further away from the central canal in the ventral or the ventrolateral direction. The CSF-cNs dendritic protrusions were clearly seen in the lumen of the central canal. Cats and macaque monkeys shared a similar expressing pattern,

where the cell bodies of PKD2L1-positive neurons were very weak (in cat) or non-visible (monkey) while the heads of the dendritic protrusions were clearly visible and closely attached to or embedded in the wall of the central canal. Due to the weak staining of the cell bodies, there were not distally distributed PKD2L1-positive cells detected in cats and monkeys. Our results indicate that intrinsic sensory neurons are conserved throughout non-mammalian and mammalian vertebrates and may play an essential role in regulating motor, sensory and autonomic functions in reaction to the internal environmental changes.

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Poster

629. Spinal Cord

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Topic: D.01. Somatosensation

Support: NIDA Grant R01DA035931
College of Pharmacy of the University of Minnesota

Title: Decarboxylated arginine (agmatine) attenuates NMDA-stimulated calcium signaling in spinal cord dorsal horn

Authors: *T. XIE[1], C. D. PETERSON[2], G. L. WILCOX[3], L. VULCHANOVA[2], C. A. FAIRBANKS[4];
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Abstract: N-methyl-D-aspartate receptors (NMDARs) in the spinal cord dorsal horn are a central component of maladaptive neuroplasticity underlying the initiation and maintenance of chronic pain, including pain of neuropathic origin. Our previous studies have demonstrated that the intrathecal administration of agmatine, an endogenous decarboxylated form of L-arginine that preferentially antagonizes GluN2B-containing NMDARs, inhibits and reverses the pain behaviors in animal models of neuropathic pain. In this study, we investigated the spinal modulatory effect of agmatine on NMDA-stimulated calcium transients and hypothesized that agmatine dose-dependently inhibits N-methyl-D-aspartate (NMDA)-stimulated calcium transients in mouse spinal cord dorsal horn, similar to other NMDAR antagonists.
Female and male ICR mice (4-6 weeks, n=3-4 per drug) were perfused before spinal cord extraction and ex vivo spinal slices were loaded with the calcium indicator dye Fluo-4. Intracellular Ca^{2+} was visualized by single plane two-photon microscopy and NMDAR antagonists were applied to spinal cord slices (APV, ifenprodil, and agmatine). Time-lapse of images were acquired and the peak amplitude of Fluo-4 fluorescence intensity were analyzed by

Student's t-test comparing cells' baseline NMDA response to the NMDA responses with applied antagonist.
With 50 µM APV application, NMDA-responsive cells showed significantly attenuated NMDA-stimulated calcium transients (n=3, P< 0.01). Similarly, 10 mM agmatine significantly decreased the NMDA-stimulated Ca^{2+} transients in all NMDA-responding cells (n=3, P< 0.005). APV (2, 10, 50 µM) and ifenprodil (30, 100, 300 µM) both concentration-dependently attenuated the NMDA-stimulated calcium transients. Agmatine (1, 3.3, 10 mM) also showed concentration-dependent inhibition of the NMDA-stimulated calcium transients. The three antagonists, APV, ifenprodil, and agmatine, did not exhibit significant sex differences in the attenuation of NMDA-stimulated Ca^{2+} response.
We observed that APV and ifenprodil significantly attenuated the intracellular NMDA-stimulated Ca^{2+} signals, indicating that the NMDA-stimulated calcium transients' assay is specific to NMDARs. Agmatine's concentration-dependent inhibition of NMDARs-stimulated calcium transients suggests that agmatine is an effective antagonist of NMDARs in the spinal cord dorsal horn, which is consistent with our previous electrophysiological and neuropharmacological research demonstrating that agmatine is an effective inhibitor of NMDARs in the spinal cord dorsal horn.

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Poster

629. Spinal Cord

Location: SDCC Halls B-H

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Topic: D.02. Somatosensation – Pain

Support: Miguel Alemán Grant

Title: Decreased activity of vglut2-c fibers decrease neuropathic pain

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Abstract: DECREASED ACTIVITY OF VGLUT2-C FIBERS DECREASE NEUROPATHIC PAIN*Amalia Alejo-Martínez[1-2], Erwin Chiquete-Anaya[2], Francisco Javier López-Muñoz[1], José de Jesús Aceves Buendía[21]Department of Pharmacobiology CINVESTAV Sede Sur, [2]Department of Neurology and Psychiatry, INCMNSZPain prevents tissue damage but can also incapacitate organisms when it is chronic. Noxious stimuli are detected by sensory afferents such as C fibers that express vGluT2 and can be expressed by peptidergic and non-peptidergic C-fibers whose axons project to laminae I and II of the spinal

dorsal horn. vGluT2 plays an important role in the physiopathology of pain because its expression increases in the spinal cord after nerve injury. NK1 neurons in the superficial laminae receive vGluT2 input from C-fibers. These neurons are important in the transmission of mechanical allodynia induced by inflammatory or neuropathic pain. **Hypothesis:** Transdermal hyperpolarization of vGluT2-C fibers with optogenetics will decrease the excitability of NK1-vGluT2^{+} neurons in the spinal dorsal horn (SDH) and will decrease neuropathic pain. **Objective:** The aim of this study was to determine if the vGluT2-C fibers send their projections to a vGluT2^{+} neuron in the SDH and hyperpolarize this input with transdermal inactivation in a neuropathic pain model. **Method**: vGluT2::Cre mice were crossed with Ai35 transgenic mice to constitutively express Arch-GFP in vGluT2 neurons. Their right sciatic nerve was ligated, and fourteen days after, noxious responses were assessed using the von Frey test and the CPP paradigm with light stimulation. In the CPP paradigm, after the first 15 min of free movement to record the basal period, for the next 45 min with yellow (560 nm) light, Arch-vGluT2 neurons were stimulated to inactivate C-fibers. Mice were perfused and transverse slices of 30 μm-thick were cut using a microtome. For immunostaining, slices were incubated with 1:250 NK1R antibody overnight washed three times before incubation with secondary antibody (Cy5; 1:1000) and then they were mounted. **Results:** The von Frey test showed that CCI group developed mechanical allodynia and hyperalgesia, and Arch activation produced CPP (optoanalgesia). Histological results showed that NK1 neurons are vGluT2^{+} in the superficial laminae of the SDH. **Conclusions:** Control of neural excitability in DRG-VGLUT2 neurons or their projections prevents pain sensitivity in an experimental neuropathic pain model through inactivation of vGluT2 neurons projecting to the SDH.

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Poster

629. Spinal Cord

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Topic: D.02. Somatosensation – Pain

Support: CIHR Grant FDN-159906
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Title: Sex differences in Cl^{-} homeostasis in c-fiber primary afferent terminals in the spinal dorsal horn.

Authors: *R. HAZRATI, F. WANG, I. KERAMIDIS, A. GODIN, Y. DE KONINCK; CERVO brain research center, Laval Univ., Quebec City, QC, Canada

Abstract: Introduction: Intracellular Cl^- concentration ([Cl^-]i) is high in primary sensory neurons due to the activity of the Na^+-K^+-Cl^- cotransporter 1 (NKCC1), causing greater Cl^- accumulation than typically seen in CNS neurons. Consequently, central terminals of primary afferents in the spinal dorsal horn experience depolarization upon activation of $GABA_A$ receptors ($GABA_AR$). Thus, regulation of $[Cl^-]_i$ in these terminals may significantly affect transmitter release. Determining the exact $[Cl^-]_i$ in C-fiber terminals is pivotal to understand sensory processing. Methods: To image $[Cl^-]_i$ we used the genetically-encoded ratiometric Cl^- sensor, superclomeleon, which was virally transduced selectively in C-fibers in SNS-Cre male and female mice. We conducted 2-photon imaging in acute spinal cord slices and acutely delaminated spinal cord in anesthetized 2, 4, and 6 months-old SNS-Cre mice. The $GABA_AR$ agonist and antagonist muscimol and bicuculline, respectively, as well as the NKCC1 antagonist bumetanide, were used to modulate $[Cl^-]_i$ in afferent terminals in the dorsal horn. NKCC1 protein and mRNA levels in the dorsal root ganglia were evaluated with western blot and RNAScope. Results: We found that $[Cl^-]_i$ in C-fibers was significantly higher in different age males than females. Bumetanide significantly decreased $[Cl^-]_i$ in males but not in females indicating a more substantial contribution of NKCC1 in maintaining higher $[Cl^-]_i$ in males. Bicuculline increased significantly $[Cl^-]_i$ in C-fibers in females but not in males. NKCC1 protein and mRNA were also significantly lower in females than males, consistent with the functional data.Conclusion: Presynaptic inhibition appears to be under distinct control by GABAergic inhibition between sexes, which should be taken into consideration in future studies.

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Poster

629. Spinal Cord

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Title: C5a / C5aR1 signaling in spinal mechanisms of neuropathic pain

Authors: K. AGASHKOV[1], A. KEYES[3], S. ROMANENKO[1], O. HALAIDYCH[1], Y. ANDRIANOV[1,3], N. V. VOITENKO[4,5], Y. M. USACHEV[3], ***P. BELAN**[2,5];
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Abstract: Recent studies suggest that neuropathic pain is associated with a robust upregulation of complement effectors in the spinal cord, which ultimately leads to production of a highly active complement product, C5a. Here, we have elucidated C5a-dependent spinal mechanisms that contribute to the development of neuropathic pain induced in mice by spared nerve injury (SNI). First, pharmacological or genetic inhibition of C5a receptors (C5aR1) decreased mechanical allodynia in SNI mice. This analgesic effect strongly suggests that C5a/C5aR1 signaling plays an important role in the development and maintenance of neuropathic pain following SNI. Next, we generated a mouse line expressing a fluorescent reporter of Ca^{2+} concentration, GCaMP5G-TdTomato, in microglia. Multiphoton Ca^{2+} imaging in an *ex vivo* intact spinal cord preparation of these mice demonstrated that C5a application induced rapid $[Ca^{2+}]_i$ increases in superficial dorsal horn microglia in a dose-dependent manner, which were blocked by co-application of a C5aR1 competitive antagonist, PMX205. These increases seem to result in the release of mediators affecting neuronal activity. Indeed, C5a application significantly increased the number of action potentials evoked by the saturating dorsal root stimulation in both unidentified and spinoparabrachial projection dorsal horn neurons. C5a application also induced an initiation of burst firing and an increase in the frequency of spontaneous firing in some neurons. Surprisingly, the afferent synaptic input was not significantly changed by the application. At the same time, C5a produced a significant decrease in the threshold of action potential generation in a subpopulation of dorsal horn neurons, which further enhances the spinal cord output to supraspinal structures. Overall, these findings demonstrate that C5a/C5aR1 signaling results in an increased intrinsic excitability of dorsal horn neurons likely via microglial activation and plays an important role in SNI-induced neuropathic pain.

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Poster

629. Spinal Cord

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Title: Spinal mu-opioid receptor expressing neurons contribute to neuropathic pain

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Abstract: Pharmacological administration of mu-opioid receptor (MOR) agonists by the intrathecal or epidural route produce profound inhibition of acute pain. These are mediated by inhibitory G-protein coupled MORs located on the central terminals of primary afferent nociceptive neurons as well as on MOR-expressing interneurons (MOR-INs) within the dorsal horn of the spinal cord. However, the contribution of MOR-INs to chronic pain is poorly understood. To fill this gap, we coupled the use of MOR^{Cre} transgenic reporter mice with whole cell patch clamp electrophysiology to evaluate the neuronal activity of MOR-INs in a mouse model of traumatic nerve injury. We also used a chemogenetic approach to activate or inhibit MOR-INs, followed by the assessment of behavioral signs of neuropathic pain. Immunochemistry revealed MOR-IN expression in both excitatory interneurons (TLX3+) and inhibitory interneurons (PAX2+). Electrophysiological studies of MOR-INs revealed four firing patterns in response to steady state depolarizing current injection: delayed, transient, phasic and tonic. These results indicate the heterogeneity of MOR-INs in the spinal dorsal horn. Spared nerve injury (SNI) reduced *oprm1* mRNA expression and responsiveness of MOR-INs to DAMGO. SNI increased the intrinsic excitability and spontaneous synaptic activity of MOR-INs neurons. SNI increased light brush-induced Fos expression in MOR-INs. These studies indicate that SNI increases the excitability of MOR-INs. Chemogenetic activation of MOR neurons in uninjured mice decreased withdrawal threshold to mechanical stimulation, while chemogenetic inhibition reduced SNI-associated mechanical and cold allodynia. Taken together, nerve injury sensitizes an important pro-nociceptive population of MOR-INs, which contribute to behavioral signs of neuropathic pain. Altering the activity of spinal MOR-INs may be a potential approach to treat chronic pain. Our future studies aim to dissect spinal MOR interneurons populations (excitatory vs inhibitory) to better understand their contribution to dorsal horn pain circuitry and opioid analgesia.

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Poster

629. Spinal Cord

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 629.13

Topic: D.02. Somatosensation – Pain

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Title: A Short Course Of Morphine Early After Peripheral Nerve Injury Induces Multi-Week Post-Morphine Potentiation Of spinal Cord Dorsal Horn Laminae I/II Excitability in Response To Later Thermal Challenge

Authors: J. B. BALL[1], S. M. GREEN-FULGHAM[1], I. R. C. ROCHA[1,2], M. HARLAND[1], M. R. FINCH[1], I. SIDDIQUE[1], **M. CHACUR**[1,2], *L. R. WATKINS[1];
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Abstract: Opioids remain a front line treatment for pain after trauma, including after peripheral nerve injury. However, our group has shown that a short, 5 day course of opioids after neuropathic pain is established INCREASES allodynia for months after cessation of opioids. This increased pain is due to the recognition of opioids by innate immune receptors, such as Toll-like receptor 4, that recognize Damage Associated Molecular Patterns (DAMPs), driving an inflammatory response. Here we show that a 5 day course of morphine, starting 10 days after nerve injury in male rats, both enhances allodynia and excitability of nociceptive neurons of the dorsal horn. In order to assess changes in neuronal excitability in the lumbar spinal cord, rats were given a unilateral chronic constriction injury (CCI) of the sciatic nerve at mid-thigh level, followed by a 5 day course of twice daily 5 mg/kg s.c. morphine starting at day 10 after nerve injury. After the morphine group had developed significantly enhanced allodynia, measured by Von Frey test (5 wk after cessation of morphine), rats were lightly anesthetized (isoflurane) and the ipsilateral paw subjected to thermal challenge via submersion in hot (52C) water for 10 rounds of 20 second immersion every 2 minutes. Rats were then sacrificed to quantify immediate early gene activation for immunohistochemistry, quantified using Bitplane's IMARIS program. Our results replicated our prior publications that 5 days of s.c. morphine after nerve injury enduringly increases allodynia. Further, we report that this morphine regimen enhances excitability of neurons in lumbar spinal dorsal horn (laminae I/II) , as measured by increases in immediate early gene cFOS and pCREB after thermal challenge. Our prior work documented that morphine, given after establishment of neuropathic pain, enhances post-trauma allodynia. The new data presented here add that amplification of pain behavior is associated with enhanced dorsal horn neuronal excitability in response to subsequent nociceptive thermal stimuli. These changes in pain processing may undermine the ability of opioids to provide sustained pain relief and a possible mechanism by which opioid use may worsen underlying chronic pain. A new understanding of opioid mechanisms may lead to the development of new drug therapies that may help reduce pain chronicity and/or reduce tolerance to opioid analgesics.

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Poster

629. Spinal Cord

Location: SDCC Halls B-H

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Program #/Poster #: 629.14

Topic: D.02. Somatosensation – Pain

Support: Conacyt, A1-S-40015
SEP-Cinvestav 269
SEP-Cinvestav 127

Title: Sex dependent pronociceptive role of the spinal alpha$_6$ gaba$_a$ receptor in neurophatic rats

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Abstract: Sex dependent pronociceptive role of the spinal α6-gabaa receptor in neurophatic rats
Morales-Moreno Ciciolil[1], García Guadalupe[1], Granados-Soto Vinicio[2], Murbartián Janet[1]
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AbstractPrevious studies have shown that extra synaptic $GABA_A$ receptors play an important role in modulation of neuron hyperexcitability in rodents. Extra synaptic α_6-containing $GABA_A$ (α_6-$GABA_A$) receptors are expressed in the trigeminal ganglion and spinal cord, which are essential sites for pain process. Also, positive allosteric modulators (PAMs) of the α_6-containing $GABA_A$ receptor reduce migraine-type and trigeminal neuropathic pain in mice and rats. On the other hand, sexual hormones can regulate the function and expression of extra synaptic $GABA_A$ receptors, including α_6-containing $GABA_A$ receptors. However, the role of spinal α_6-containing $GABA_A$ receptors in neuropathic pain in both sexes has not been investigated. Thus, the purpose of this study was to determine whether sex as well as sex hormones participate in the effect of α_6-$GABA_A$ receptors in neuropathic rats. L5 and L6 spinal nerve ligation reduced withdrawal threshold in Wistar rats, which was interpreted as tactile allodynia. Intrathecal administration of the α_6-$GABA_A$ receptor PAM PZ-II-029 (1-10 nmol, 14 days after nerve injury) reduced tactile allodynia, in a dose-dependent manner, in female, but not in male rats. Of note, ovariectomy (ovx) abated the antiallodynic effect of PZ-II-029 (10 nmol) in female rats. Accordingly, 17-β-estradiol (20 µg/Kg daily for 14 days) fully restored the antiallodynic effect of PZ-II-029 in ovx female rats. Intrathecal administration of ICI-182,780 (0.5 µg, estrogen receptor (ER) α and β antagonist) or MPP (0.5 µg, selective ERα antagonist) prevented the antiallodynic effect of PZ-II-029 (10 nmol) in estradiol-treated neuropathic ovx female rats. Our data suggest that spinal α_6-$GABA_A$ receptors exert a sex-dependent antiallodynic effect in neuropathic rats. Moreover, results suggest that estradiol and ERα are necessary to activate spinal α_6-$GABA_A$ receptors in female rats. PAMs of α_6-$GABA_A$ receptors could be useful to treat neuropathic pain in women.
Grants: Conacyt, A1-S-40015 (VG-S), SEP-Cinvestav 269 (JM) and 127 (VG-S)

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Poster

629. Spinal Cord

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant 1R01NS102850
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Title: Epileptiform bursting in spinal dorsal horn circuits drives ectopic spiking in primary afferents: putative role in sensory dysfunction after spinal cord injury

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Abstract: Spinal cord injury (SCI) leads to profound sensory dysfunction that commonly includes neuropathic pain. Prominent mechanisms linked to the development of neuropathic pain after SCI are: 1) increased spontaneous activity in afferent cell bodies in dorsal root ganglia (DRG), and 2) disinhibition in dorsal horn circuits that support allodynia. To probe central and peripheral plasticity after SCI, we recorded alterations in sensory function in an adult *ex vivo* intact spinal cord preparation and a truncal skin-nerve preparation, respectively. Recordings in the spinal cord preparation were obtained from naïve (n=2), sham (n=7), and lower thoracic contusion SCI (n=12) populations. The skin-nerve preparation data includes recordings from naïve (n=2) and SCI (N=3) mice. Data was analyzed using Spike2, as well as custom Matlab and R scripts. We show that SCI and sham, but not naïve, spinal cords exhibit spontaneous bursts. Bursts in SCI cords occurred at significantly greater frequency and amplitude than sham cords. These bursts were more likely to be epileptiform, synchronous, and associated with ectopic antidromic afferent bursts across several segments in SCI preparations. Bursting afferents conduction velocities suggest preferential recruitment of slower-conducting afferents (not Aβ). 4-aminopyridine (4-AP) induced synchronous epileptiform bursting in sham and naïve cords but did not alter the already epileptiform bursting seen after SCI. In all cases, spontaneous bursting events were greatly depressed or fully blocked by $GABA_A$ receptor antagonists, supporting critical involvement of GABAergic inhibitory neurons (GINs), presumably those with presynaptic axo-axonic synapses on intraspinal afferents. To examine whether antidromically traveling bursts could influence air-evoked low-threshold mechanoreceptor (LTMR) recruitment, we reproduced recorded antidromic afferent burst spiking events by delivering antidromic stimulus trains to dorsal cutaneous nerves in the truncal skin-nerve preparation (n=3). We observed that antidromic bursting led to lasting (several minutes) but reversible depression of LTMR recruitment following antidromic stimuli that recruited slower (Aδ and C), but not fast-conducting (Aβ) afferents. These results suggest that, after SCI, GINs are key players in emergence of a bursting circuitry capable of synchronizing across multiple spinal segments and powerfully driving presynaptic spiking in primary afferents. Whether ectopic afferent spiking supports reentrant excitatory drive that contributes to bursts, as well as how these bursts are associated with sensory dysfunction, are currently being determined.

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Poster

629. Spinal Cord

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Topic: D.02. Somatosensation – Pain

Support: Korean government Ministry of Science 2015M3D6A1065094
Korean Ministry of Health and Welfare HI15C3518

Title: Combination gene delivery reduces spinal cord pathology in rats with peripheral neuropathic pain

Authors: H. JI[1], ***K.-R. KIM**[1], J.-J. PARK[1], J. LEE[1], Y. SIM[2], J. SHIN[1], S.-O. HONG[1], H. CHOI[3], S. KIM[1];
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Abstract: Peripheral neuropathic pain (NP) is caused by peripheral nerve injury, however it is not just a peripheral nervous system disease. It causes abnormalities in the central nervous system as well as the peripheral nervous system. The pathological phenomena such as hyperactivation of sensory neurons and inflammation are observed in both the dorsal root ganglion (DRG) and spinal cord (SC). The pain signals originated from periphery are transmitted to the brain through the SC, and the signals could be modulated by pathologically changed SC conditions. Therefore, the modulation of SC pathology is important for peripheral NP treatment. In this study, we investigated the effects of KLS-2031 (recombinant adeno-associated viruses expressing glutamate decarboxylase 65, glial cell-derived neurotrophic factor and interleukin-10) delivered to the DRG on the aberrant neuronal excitability and neuroinflammation in the SC of rats with NP. To deliver KLS-2031 to DRGs, we utilized a TF injection method, which is widely used to deliver drugs into the epidural space, in rats with spared nerve injury (SNI) showing the symptoms of NP such as mechanical allodynia. To examine the change of pain signal transmission in SC under the NP state, we measured the excitatory and inhibitory postsynaptic currents of substantia gelatinosa (SG, lamina 2) neurons in the dorsal horn region of the SC in rats with SNI 4 weeks after TF injection of KLS-2031. Results showed that KLS-2031 administration reduced excessive excitatory transmission and recovered inhibitory synaptic signal in SG neurons. To mimic the physiological condition of pain transmission from peripheral nerve to CNS, we performed *in vivo* extracellular recording of wide dynamic range (WDR) neurons to physiologically explore the signals of peripheral tissues and evaluate the degree of in vivo pain. The *in vivo* hypersensitivity of WDR neurons in SNI rats was restored by TF injection of KLS-2031. Furthermore, we assessed the effect of KLS-2031 on neuronal inflammation in the SC by the activation of microglia and astrocytes using ionized calcium-binding adaptor molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP) as microglia and astrocyte markers, respectively. KLS-2031 mitigated neuroinflammation in the SC by regulating microglia and astrocytes. Collectively, KLS-2031 effectively suppressed the central sensitization of pain

signals and inflammation in the SC of a NP model and offers a potentially new therapeutic approach for NP.

Disclosures: **H. Ji:** A. Employment/Salary (full or part-time):; Kolon Life Science 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.; Korean government Ministry of Science and ICT, Korean Ministry of Health and Welfare. **K. Kim:** A. Employment/Salary (full or part-time):; Kolon Life Science 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.; Korean government Ministry of Science and ICT, Korean Ministry of Health and Welfare. **J. Park:** A. Employment/Salary (full or part-time):; Kolon Life Science 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.; Korean government Ministry of Science and ICT, Korean Ministry of Health and Welfare. **J. Lee:** A. Employment/Salary (full or part-time):; Kolon Life Science 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.; Korean government Ministry of Science and ICT, Korean Ministry of Health and Welfare. **Y. Sim:** A. Employment/Salary (full or part-time):; Yungjin Pharm. **J. Shin:** A. Employment/Salary (full or part-time):; Kolon Life Science 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.; Korean government Ministry of Science and ICT, Korean Ministry of Health and Welfare. **S. Hong:** A. Employment/Salary (full or part-time):; Kolon Life Science 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.; Korean government Ministry of Science and ICT, Korean Ministry of Health and Welfare. **H. Choi:** A. Employment/Salary (full or part-time):; Kolon Industries. **S. Kim:** A. Employment/Salary (full or part-time):; Kolon Life Science 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.; Korean government Ministry of Science and ICT, Korean Ministry of Health and Welfare.

Poster

629. Spinal Cord

Location: SDCC Halls B-H

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Program #/Poster #: 629.17

Topic: D.02. Somatosensation – Pain

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Stanford NeuroChoice

Title: An inflammatory injury model enhances spino-periaqueductal gray projection neuron excitability, and may lead to lasting circuit alterations within the periaqueductal gray

Authors: *C. L. BREWER, J. A. KAUER;
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Abstract: Prior work has demonstrated that modeling inflammatory injury via low-frequency stimulation of high-threshold sensory afferents selectively potentiates these excitatory synapses onto spinal projection neurons targeting the periaqueductal gray (spino-PAG PNs). We sought to characterize this plasticity by identifying the underlying peripheral sensory population and effects on the output of spino-PAG PNs. We chose to isolate and control the activation of TRPV1-expressing sensory neurons, which include a large proportion of high threshold afferents, using adolescent, mixed-sex mice expressing channelrhodopsin in TRPV1-lineage (TRPV1+) neurons. Spinal PNs were back-labeled from the PAG using diI. Whole-cell patch-clamp recordings revealed that stimulation (via 1 ms 470 nm LED pulse) of TRPV1+ afferents induced burst firing in most spino-PAG PNs. We modeled inflammatory injury by stimulating TRPV1+ fibers at 2 Hz for 2 min (LFS), as peripheral inflammation induces 1-2 Hz firing in high-threshold C fibers. LFS of TRPV1+ afferents enhanced the synaptic and intrinsic excitability of spino-PAG PNs—eliciting a stable (≥2 hours) increase in the number of action potentials (APs) within a TRPV1+ fiber-induced burst (n=10, p=0.029), while decreasing the intrinsic AP threshold (n=14, p=0.015) and membrane resistance (n=14, p=0.0003) of the PNs. Further experiments revealed that this plasticity was dependent on postsynaptic G protein-coupled signaling, NMDA receptor activation, and TRPV1+ afferent input. Taken together, this work suggests that inflammatory injury persistently enhances the output of the spino-PAG pathway. Additional work is needed to characterize the downstream effects of this plasticity in the PAG and define the function of this pathway in nociception. We are currently using mice expressing excitatory opsins in ascending spinal afferents while performing patch clamp recordings in the PAG. Our preliminary results in the ventrolateral PAG suggest that LFS of ascending spinal axons depresses these excitatory inputs. Furthermore, we find that glutamatergic spinal afferents synapse onto μ opioid receptor-expressing PAG cells. These early results suggest that spinal inputs to the PAG could play an integral role in this midbrain region's modulation of somatosensation and opiate sensitivity — warranting further exploration of the organization and function of these neural pathways.

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Poster

629. Spinal Cord

Location: SDCC Halls B-H

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Topic: D.02. Somatosensation – Pain

Support: CIHR Foundation Grant FDN 159906
SFARI Pilot and Research Award

Title: A novel enhancer of the K+-Cl- cotransporter 2 with improved pharmacokinetics for in vivo applications

Authors: I. PLASENCIA-FERNANDEZ[1], M. GAGNON[2], S. TRIPATHY[3], G. ATTARDO[3], I. KIANICKA[3], M. PAQUIN[3], P. CUSSON[3], M. JACOB-WAGNER[3], D. VILLENEUVE[3], J. A. COULL[3], A. G. GODIN[1], *Y. DE KONINCK[1];
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Abstract: The regulation of Cl^- levels is essential for inhibition between neurons. The maintenance of this Cl^- homeostasis in adult neurons is controlled by the K^+ Cl^- cotransporter 2 (KCC2) and its downregulation results in a loss of effective inhibition in the central nervous system. There is accumulating evidence that demonstrates how reduced KCC2 activity is a common factor among several neurological disorders characterized by an excitatory/inhibitory imbalance. Therefore, the design of new drugs that specifically enhance KCC2 activity opens new avenues for the study of these disorders and for neurological treatment development. Using an assay for high-throughput screening led to the identification of a new family of pyrimidine compounds that reduce intracellular Cl^- concentration. Optimization of the compounds resulted in a KCC2 selective activator as confirmed by different in vitro cellular assays, neuronal tissue as well as in vivo evaluations. This molecule was able to enhance Cl^- extrusion in a dose and time-dependent manner only in cells expressing KCC2. Moreover, in rat spinal dorsal horn neurons the compound caused hyperpolarization of the GABA reversal potential after KCC2 downregulation that had resulted from TrkB receptor stimulation or peripheral nerve injury. This molecule was characterized by a good oral bioavailability, pharmacokinetic profile, and blood-brain barrier permeability making it well suitable for in vivo applications. We found that oral administration of the compound was able to reduce hyperalgesia in a rat model of neuropathic pain after a single dose and induced a sustained analgesia with repeated treatment for up to 5 days. In contrast with other analgesic treatments, we did not observe any motor impairments associated with the treatments. Toxicological studies in rats and dogs performed blindly by an independent contract research organization revealed no observable effect at doses of 300 mg/kg on clinical condition, body weight, food consumption, hematology, coagulation, clinical chemistry, urinalysis, organ weight, or histopathology. Therefore, this molecule appears as a KCC2 enhancer with optimal drug-like properties making it a valuable therapeutic candidate.

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Poster

629. Spinal Cord

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Program #/Poster #: 629.19

Topic: D.01. Somatosensation

Support: 1R41NS120748

Title: Sensitrak: automated assessment of forelimb sensation in rats

Authors: *A. RAMAMURTHY* [1], D. YOO* [1], C. SANCHEZ [2], A. M. SLOAN [2], J. CARMEL [1]; [1]Columbia Univ. Med. Ctr., New York, NY; [2]Vulintus Inc., Westminster, CO

Abstract: Somatosensory function is routinely measured in the clinic for patients with neurological injuries, and discrimination ability is a strong predictor of dexterity and upper limb function. For preclinical research, however, there are currently no turnkey systems available that can administer analogous volitional measures of tactile or proprioceptive function in rodent models. To meet this need, our team is developing the SensiTrak system, a suite of automated, operant behavioral tasks for rats and mice which assess discrimination thresholds for vibration, texture, and proprioceptive modalities. Here we present baseline psychophysical threshold from non-injured rats for the first three tasks developed for the system: a Go/NoGo vibrotactile detection task, a two-alternative forced-choice (2-AFC) texture discrimination task, and a 2-AFC proprioception discrimination task. All three tasks enable the ascertainment of a classic sigmoidal sensitivity function. Once the baseline is quantified, SensiTrak measurements can detect and quantify impairments from neurological injuries; rats trained on the vibrotactile task were tested prior to and following two models of transient somatosensory impairment. In the first model, we administered injections of the local anesthetic bupivacaine to the wrist, temporarily desensitizing the paw, and observed significant increases in vibrotactile detection thresholds, which were fully recovered after 12 hours. In the second model, rats underwent a lesion to the dorsal column medial lemniscal (DCML) pathway, which typically results in a loss of vibration sense in the upper limb. Rats tested on the vibrotactile task following DCML lesion showed significant impairment up to 3 weeks post-injury, with thresholds returning to baseline at ~5 weeks post-injury. Development of SensiTrak is ongoing, and continuing studies will test the sensitivity of the texture and proprioceptive discrimination tasks for measuring injury-associated impairment, with plans to extend behavioral testing to mice.

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Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.01

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 1R01DA035484

Title: Pain-evaluative states influence brain representations of negative affect

Authors: *M. CEKO[1], C.-W. WOO[2], M. LÓPEZ-SOLÀ[3], T. D. WAGER[4];
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Abstract: Negative affect is encoded in the brain as a combination of stimulus-type-specific and generalized brain systems. Representations of aversive stimuli in these systems may be influenced by task context. An often-neglected type of context is the type of judgment made about a stimulus. We can attend to or away from a stimulus; but we can also attend to (and evaluate) particular ***qualities***, including affective (i.e., aversiveness) and sensory-discriminative (i.e., stimulus intensity). Here we tested how manipulating instructions to evaluate affective vs. sensory-discriminative qualities of a painful stimulus influences pain-specific and generalized negative affect brain representations. FMRI data were acquired in 34 healthy participants (age 25 ± 8 [mean ± SD]; 21 males), receiving 2 levels of painful pressure in 3 randomized conditions: focusing on stimulus aversiveness (Affective Eval), intensity of applied pressure (Sensory Eval), or passively experiencing the stimulus (Passive Responding). FMRI data were analyzed using SPM and custom Matlab tools. Brain models predictive of stimulus-type-specific (mechanical pain, thermal pain, negative auditory, negative visual) and generalized negative affect (Ceko 2022) were applied to individual contrast maps to compare the conditions. A meta-analytic emotion regulation pattern (Buehle 2014) was applied to test the hypothesis that emotion regulation maps more closely to the affective vs. sensory evaluation. Significance was tested using a 2-sided 1-sample t-test. We previously observed activation during Affective Eval in midline regions linked to internal evaluative processing and emotional appraisal, and activation during Sensory Eval in lateral regions linked to external attention and stimulus discrimination. Here, general and mechanical pattern showed a significant response during Passive Responding to mechanical pain ($p = .003$ and $p = .0007$, respectively), but the other patterns did not ($ps > .1$), showing sensitivity and specificity of affect models. General and mechanical patterns were significantly more activated by Affective Eval vs. Passive and Sensory Eval vs. Passive ($p = .02$ and $p = .01$, respectively). The emotion regulation pattern was significantly activated in Affective, but not Sensory Eval, vs. Passive ($p = 0.01$ vs $p = 0.3$, $p = 0.07$ for Sensory vs.

Affective) in line with work showing that regulation has a stronger effect on affective vs. sensory properties of negative affect. These findings underline the recruitment of core affect systems during internal evaluative and regulatory processes, and of early sensory discriminative brain systems during the evaluation of external states.

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Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

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Program #/Poster #: 630.02

Topic: D.02. Somatosensation – Pain

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2021M3E5D2A01022515
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2E30410-20-085

Title: The Landscape of Pain Prediction: A Systematic Review and Benchmarking Analysis

Authors: *D. LEE, S. LEE, C.-W. WOO;
Ctr. for Neurosci. Imaging Res., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Neuroimaging-based pain biomarkers, by applying machine learning techniques to neuroimaging data, have shown the potential to decode the level of pain intensity or to diagnose clinical pain conditions. However, the systematic evaluation of how different modeling options influence the model performance has yet to be done. Here, we provide results from a systematic literature survey and a benchmarking analysis. For the literature survey, we conducted a survey of published articles that included neuroimaging-based predictive modeling of pain (the total number of surveyed studies, N = 57) and compared the model performances on the following modeling variables—the levels of data (e.g., TR-, trial-, run- and condition-level), spatial scales (voxels to whole-brain), idiographic vs. population models, and sample sizes. For the benchmarking analysis, we collected a large-scale fMRI heat pain dataset (participants N = 124). With the dataset, we trained and tested predictive models of pain and compared the model performances across modeling variables that we examined in the survey. The survey and benchmarking analysis results showed that the classification and prediction model performances were higher when the models were trained on the data averaged over more trials and when the models included more brain regions. In addition, the benchmarking analysis showed that the model performances became higher when the models were trained on larger sample sizes. These results suggest that the model targets and options can have a significant impact on the model performance and serve as a benchmark for developing neuroimaging-based pain biomarkers.

Disclosures: **D. Lee:** None. **S. Lee:** None. **C. Woo:** None.

Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.03

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 5R35GM142676-02

Title: Utilizing fNIRS to explore the relationship between pain catastrophizing and neural activity in post-surgical pain: A pilot investigation

Authors: M. L. JOTWANI[1,2], C. E. LUNDE[1,3,2], G. COMPTDAER[1,2], H. GAGNON[1,2], A. WOLFSON[1,2], *Z. WU[1,2,4], C. B. SIEBERG[1,5,2,4];
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Abstract: Introduction. Chronic post-surgical pain (CPSP) is a significant public health concern affecting up to 80% of adults who have undergone surgery and is a contributor to the opioid epidemic (Wunsch et al, 2016). Little research has been conducted on neural mechanisms that result in a risk for CPSP development, and how surgery confers brain changes that result in pain chronification or increased resistance to treatment. Additionally, it is important to assess how psychological variables (e.g., pain catastrophizing, pain acceptance), which have known neurobiological underpinnings, impact brain metrics (i.e., brain activation, functional connectivity (FC)) to maintain chronic pain in patients following surgery. **Methods.** The aim of this investigation (data collection ongoing) was to utilize functional near-infrared spectroscopy (fNIRS) to explore evoked pain brain functional properties and its relation to pain catastrophizing and pain acceptance in 7 patients (age:41±20.067 years) with CPSP compared to 7 healthy controls (HCs) (age:26.71±8.420 years). Specifically, participants completed the *Chronic Pain Acceptance Questionnaire*, which measures the ability to accept pain and continue life activities in the presence of pain (McCracken, Vowles & Eccleston, 2004), and the *Pain Catastrophizing Scale*, which assesses pain-related worry (Sullivan, Bishop & Pivik, 1995) and underwent an evoked thermal pain stimulus paradigm via fNIRS**. Results.** Neuroimaging targeted the bilateral prefrontal and somatosensory cortices, regions known to be involved in pain (Yang & Chang, 2019). A two-tailed T-test showed that HCs had significantly reduced oxygenated activation in the left somatosensory cortex compared to surgical patients ($t=2.368$, $p=0.037$). Left somatosensory cortex activation was significantly positively correlated with pain catastrophizing via a Pearson Correlation ($R=0.873$, $p=0.023$) in HCs. Further, compared to HCs, surgical patients showed enhanced FC between the left medial prefrontal cortex and left somatosensory cortex ($t=2.353$, $p=0.038$). Increased prefrontal-somatosensory connectivity was significantly correlated to greater pain catastrophizing ($R=0.950$, $p<0.01$) in patients. **Discussion.** Living with CPSP impacts psychological factors like pain catastrophizing, which may be

reflected in abnormal activation and FC patterns in the somatosensory and prefrontal cortices. Our evidence suggests that these factors could be important therapeutic targets for prevention and treatment for post-surgical pain. Larger studies investigating biobehavioral underpinnings of CPSP are warranted and underway in our lab.

Disclosures: M.L. Jotwani: None. **C.E. Lunde:** None. **G. Comptdaer:** None. **H. Gagnon:** None. **A. Wolfson:** None. **Z. Wu:** None. **C.B. Sieberg:** None.

Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.04

Topic: D.02. Somatosensation – Pain

Title: Development of fNIRS based biomarkers of chronic pain

Authors: *J. KING[1], B. NEPHEW[1], E. SOLOVEY[1], A. HOWELL-MUNSON[1], P. BUSARANUVONG[2], J. POLCARI[1], J. CIROLI[1], A. BALASUBRAMANIAN[1], C. RUIZ[1]; [1]Worcester Polytechnic Inst., Worcester, MA; [2]Worcester Polytechnic Inst., Wocester, MA

Abstract: Increased prevalence of chronic pain and limitations in its treatment, including the ongoing opioid epidemic, are critical challenges in US healthcare. The development of reliable and etiologically relevant biomarkers of chronic pain would assist clinicians with both assessing and treating pain and enhance the targeting and development of interventions. Given the unavoidable subjective elements of pain sensation, including individual variation in central somatosensory perception and related cognitive and emotional processing, brain based biomarkers are particularly valuable. The present study collected functional near-infrared spectroscopy data from subjects with (19) and without (20) chronic pain during the completion of a simple locomotor task, the Timed Up and Go (TUG), and applied machine learning to identify predictors of chronic pain. Using deoxygenated hemoglobin fNIRS signals collected from subjects before, during, and after the TUG task, we categorized chronic pain vs. control subjects with 71% accuracy. When the fNIRS data were combined with 5 days of self-reported PROMIS-29 pain assessment data, the accuracy increased to 87%, which was greater that the accuracy of using the PROMIS-29 alone (82%). These results provide valuable insight on the optimal use of machine learning with fNIRS data to develop biomarkers of chronic pain, indicate that fNIRS may be an accurate predictor of chronic pain, and demonstrate that the addition of fNIRS data to PROMIS-29 assessment improves predictive accuracy.

Disclosures: J. King: None. **B. Nephew:** None. **E. Solovey:** None. **A. Howell-Munson:** None. **P. Busaranuvong:** None. **J. Polcari:** None. **J. Ciroli:** None. **A. Balasubramanian:** None. **C. Ruiz:** None.

Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.05

Topic: D.02. Somatosensation – Pain

Support: NSF DGE-1324585
NIH F31NS126012
NIH 1P50DA044121

Title: A mesolimbic valence competition model explains acute pain dynamics

Authors: *A. D. VIGOTSKY[1,2,3], R. JABAKHANJI[3,4], M. N. BALIKI[3,5,7], A. V. APKARIAN[3,4,5,6];
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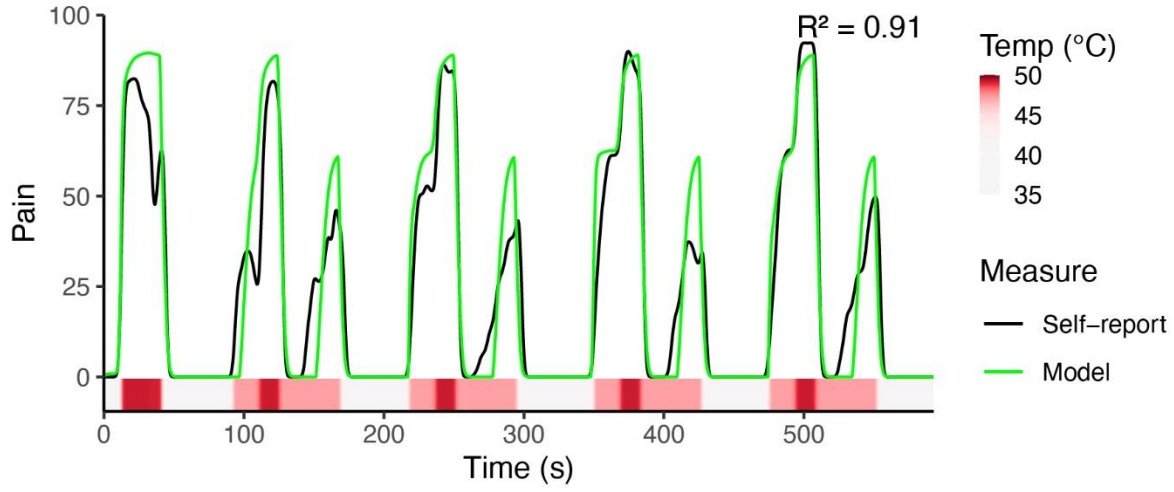
Abstract: Temporal fluctuations in the intensity of a noxious stimulus produce complex and often unintuitive changes in pain. For instance, offset analgesia is the disproportionately large decrease in pain following a slight decrease in the intensity of a noxious stimulus, dissociating the noxious stimulus from pain via a history-dependent mechanism. These complex dynamics have been captured using phenomenological models derived from system identification; however, such models are a black box and provide little insight into the neural mechanisms that give rise to acute pain's complex dynamics. Since a circuit's behavior is governed by its architecture, there is much to learn from generative, circuit motif-based models—a correctly specified model is sufficient to account for the observed data. Here we introduce a circuit-inspired model of acute pain dynamics. Our model is based on the novel hypothesis that the mesolimbic system's positive and negative reward mechanisms compete with one another. For example, when the intensity of a noxious stimulus is reduced, it is associated with a positive reward since the organism has presumably found a path to safety. Our hypothesis posits that such a positive reward would inhibit the circuits responsible for encoding negative valence, effectively preventing the experience of pain. The circuit model reflecting our hypothesis can be reduced to a pair of coupled, first-order differential equations containing between 5 and 8 interpretable parameters in total, depending on the assumptions one is willing to make about the symmetry of the positive-negative valence competition. We assessed our model's ability to capture the pain dynamics associated with onset hyperalgesia, offset analgesia, and stepwise increases with sequential offset analgesia. Our mesolimbic valence competition model captured the salient dynamics of all three tasks, lending preliminary support to our novel hypothesis. Importantly, our model also produced interpretable parameters linked to our mechanistic hypothesis of offset analgesia and other dynamical acute pain phenomena.



Disclosures: **A.D. Vigotsky:** None. **R. Jabakhanji:** None. **M.N. Baliki:** None. **A.V. Apkarian:** None.

Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.06

Topic: D.02. Somatosensation – Pain

Support: Bertha Rosenstadt Endowment Fund
NSERC Discovery Grant: RGPIN-2018-04908

Title: Delineation of the Trigeminal-Lateral Parabrachial-Central Amygdala Tract in Humans: An Ultra-High Field Diffusion MRI Study

Authors: *B. KAYA, M. MOAYEDI;
Fac. of Dent., Univ. of Toronto, Toronto, ON, Canada

Abstract: Facial pain is thought to be evolutionarily more salient than pain elsewhere in the body due to the importance of the face in social, feeding, and exploratory behaviors, amongst others. Facial pain is processed by several nociceptive circuits projecting from the trigeminal nerve to several brain regions, via the trigeminal brainstem sensory nuclear complex (VBSNC), and ascending pathways projecting to mesencephalic and thalamic targets. Recent evidence suggests that a monosynaptic circuit from the trigeminal nerve directly to the lateral parabrachial nucleus (PB_L) that bypasses the VBSNC, identified in rodents, underlies the greater unpleasantness elicited by pain in the orofacial region. The PB_L further projects to the central amygdala (CeA), which is thought to contribute to the affective component of pain. In rodents,

direct activation of this pathway elicited pain responses such as avoidance behaviors and distress vocalizations. Here, we aim to determine whether such a circuit can be resolved in the human trigeminal system using ultra-high field (7T) diffusion-weighted imaging (DWI). We performed tractography to resolve the CNV REZ-PB_L-CeA circuit. The PAG was used as an exclusion mask given dense PAG-amygdala projections and ascending trigeminal nociceptive tracts to the PAG. The basolateral amygdala (BLAT) was used as a negative control, given that we do not anticipate strong CNV REZ-PB_L-BLAT connectivity. The amygdalar seeds were imported from the Tyzska-Pauli atlas. The PBL and PAG seeds were imported from the Brainstem Navigator probabilistic atlas. Bidirectional probabilistic tractography was performed between each amygdalar nucleus (CeA and BLAT) and CNV REZ with PB_L as a waypoint with 10,000 samples per voxel for each seed, and hemisphere. Connectivity strength was based on the number of tracts between each region of interest, corrected for seed size, and compared using Wilcoxon signed-rank tests. Significance was set at a Bonferroni-corrected $p < 0.025$. Two participants were removed from the analyses due to outlying data points (2 SD outside the mean). The right CNV REZ-PB_L-CeA circuit had a significantly stronger connectivity strength than the right CNV REZ-PB_L-BLAT circuit ($T = 80$, $p = .016$, effect size (r) = 0.758). There were no significant differences between the circuits in the left hemisphere ($T= 66$, $p = .152$, $r = .451$). This exploratory study aimed to delineate the CNV REZ- PB_L-CeA circuit in humans for the first time. We report the presence of this circuit, but these results must be reproduced in a larger sample. This circuit provides a neuroanatomical substrate for the affective dimensions of orofacial pain.

Disclosures: B. Kaya: None. **M. Moayedi:** None.

Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.07

Topic: D.02. Somatosensation – Pain

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)
Unifying Neuroscience and Artificial Intelligence in Quebec (UNIQUE)
Arthritis Society and Fonds de Recherche du Québec - Santé

Title: Bundle specific white matter microstructure is altered in chronic low back pain patients and can identify subacute back pain patients transitioning to chronic pain.

Authors: *G. LITTLE[1], A. BORE[1], M. DESCOTEAUX[1], P. TÉTREAULT[2];
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Abstract: Using brain magnetic resonance imaging (MRI), chronic low back pain (CLBP) has been associated with the reorganization of the central nervous system, but little is understood about microstructural alterations underlying the transition from acute injury to CLBP. Others

have observed white matter (WM) anomalies associated with the transition to CLBP, reporting altered cortico limbic connectivity and microstructure (lower fractional anisotropy (FA)). However, previous diffusion MRI studies employ techniques that ignore the full extent of WM bundles, limiting the anatomical specificity of results. Here, we use a methodology that extracts diffusion tensor imaging (DTI) metrics from each fiber bundle to assess WM bundle microstructure alteration in patients suffering from CLBP, and to determine if these metrics can identify those who transition to CLBP.
OpenPain.org, a public imaging dataset contains four groups of individuals; healthy controls (CON, N=22, age 36 ± 8, 13 males), individuals with CLBP (N=24, age 44 ± 8, 15 males), individuals with subacute back pain (SBP) but recovered (SBPr, N=29, age 44 ± 12, 17 males) and those whose pain persisted past a year (SBPp, N=29, age 44 ± 9, 18 males). For now, only visit 1 data of all subjects was analyzed. Structural T1w (1 x 1 x 1 mm3) and diffusion MRI (2 x 2 x 2 mm3, 8 b0, 60 b=1000s/mm2) was acquired for each subject. Images were processed with the Tractoflow pipeline outputting tractograms generated using local probabilistic tracking. Then bundle segmentation was used (RecobundlesX) to output 33 WM bundles. FA was interpolated onto each bundle and averaged outputting an FA value per bundle per subject. T-tests were performed to assess FA differences per bundle ($p < 0.01$) for two contrasts; CON vs CLBP and SBPr vs SBPp.
Three bundles had lower FA in CLBP compared to CON; the left superior longitudinal fasciculus 1 (SLF 1, CON mean FA 0.40 ± 0.02, CLBP mean FA 0.39 ± 0.02), the left SLF 3 (CON mean FA 0.39 ± 0.02, CLBP mean FA 0.38 ± 0.05) and the right arcuate fasciculus (AF, CON mean FA 0.40 ± 0.02, CLBP mean FA 0.39 ± 0.03). Three bundles had lower FA in SBPp compared to SBPr, these were the left AF (SBPr mean FA 0.42 ± 0.02, SBPp mean FA 0.41 ± 0.02), the anterior portion of the corpus callosum (SBPr mean FA 0.45 ± 0.03, SPBp mean FA 0.43 ± 0.02) and left SLF 1 (SBPr mean FA 0.40 ± 0.03, SBPp mean FA 0.38 ± 0.02). These results demonstrate that the transition from subacute to chronic pain can be predicted by changes in DTI metrics derived from whole fiber bundles. Interestingly, the left SLF 1 was lower in both SBPp and CLBP groups, which suggests that FA alterations to this specific bundle relate to the likelihood of an individual to transition from SBP to CLBP.

Disclosures: **G. Little:** None. **A. Bore:** None. **M. Descoteaux:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IMEKA. **P. Tétreault:** None.

Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.08

Topic: D.02. Somatosensation – Pain

Support: NINDS - Mechanisms, Models, Measurement and Management in Pain Research (R01) Grant R01NS102415
NIH CTSA post-doctoral fellowship TL1TR002531 – Nguyen, Tyler

Title: In vivo monitoring of neuroinflammation due to mild traumatic brain injury elicits prolonged NLRP3 inflammasome activation of caspase-1 and nociplastic pain states in male and female mice.

Authors: *T. NGUYEN[1], A. G. COCHRAN[4], N. N. NGUYEN[2], J. A. SMITH[3], M. JUBOORI[5], S. TALLEY[6], E. CAMPBELL[7], F. A. WHITE[8];
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Abstract: Mild traumatic brain injury (mTBI) constitutes a major global health problem with neuropathological/behavioral sequelae which often is accompanied by long-term nociplastic pain states. Nociplastic pain is associated with prolonged inflammation often observed with the mTBI. To better understand the sequelae of inflammatory responses following single and repetitive mTBI, we assessed the degree to which the NLRP3 inflammasome contributes to the pathological response. Assembly of the NLRP3 inflammasome leads to caspase 1-dependent release of the pro-inflammatory cytokines IL-1β and IL-18. In this study, we utilized a transgenic caspase1-activated-luciferase reporter mouse which allowed us to monitor the initiation, progression, and resolution of neuroinflammation in living animals. These mice were subjected to a skull thinning cranial window preparation, and both single and repetitive controlled cortical impact injuries. We observed robust increases of caspase-1 activation within 24 hours after either single or multiple injuries which was sustained for at least one month and evident in both sexes. Stimulus-dependent tactile behavioral assessments demonstrated robust decreases in paw withdrawal threshold which lasted for almost two months in mice subjected to three mTBI events though the behavioral changes were more profound in female mice than male mice. Treatment of injured animals with MCC950, a selective inhibitor of the NLRP3 inflammasome, attenuated both the pathological inflammatory response and significantly increased paw withdrawal thresholds in both male and female mice. These data establish a model whereby the development and progression of neuroinflammatory responses associated with injury to the nervous system can be monitored and potentially provide us with a mechanistic understanding of the role of the NLRP3 inflammasome in nociplastic pain.

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Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.09

Topic: D.02. Somatosensation – Pain

Support: Sanford Institute Grant 2002703

Title: I fear your pain: the role of the amygdala in human empathy

Authors: *V. OLIVA[1], G. RIEGNER[1], L. KHATIB[1], J. DEAN[1], J. ROSS[1], C. LOPEZ[1], A. ALLEN[1], D. BARROWS[1], A. UVAROVA[1], M. REYES[1], R. FUENTES[1], A. ALEXIOU[1], D. MOSBEY[1], L. LINARES[1], W. MOBLEY[2], F. ZEIDAN[1];
[1]Dept. of Anesthesiol., [2]Dept. of Neurosciences, Univ. of California San Diego, La Jolla, CA

Abstract: Empathy is characterized as the ability to share one's experience. Recent findings indicate that the rostral anterior cingulate (rACC) and insular cortices play a role in empathy. For example, insular lesions lead to less empathetic behaviors. Further, neuroimaging studies revealed that viewing and/or mentalizing one's romantic partner (RP) in pain produces higher rACC and anterior insula activation. However, these studies employed blood oxygen level dependent fMRI that may not comprehensively capture tonic empathetic responses to pain. The proposed pilot project (targeted sample size = 45; data collection ongoing) used a novel experimental paradigm that continuously acquired cerebral blood flow (CBF) changes with arterial spin labeling (ASL) fMRI while a female volunteer viewed her RP and a "stranger" receive noxious heat. Based on prior work, we predicted that higher empathy would be associated with higher rACC and anterior insula activity.

Fifteen healthy females (mean age = 32 years) were administered a noxious "heat series" (ten, 8s 48°C plateaus; 240 seconds; left forearm) during ASL fMRI (3T GE MR750). The subject then viewed, with an MRI-compatible mirror, a "stranger" (laboratory technician) and then her RP receive the same "heat series" in the MRI room. Visual analog scale (VAS) ratings for empathy (0 = "not unpleasant" to 10 = "most unpleasant imaginable") and pain intensity (0 = "no pain" to 10 = "worst pain imaginable") were collected from all participants after each scan.

Subject level neuroimaging analyses (FSL 6.0.4) compared CBF corresponding to 1) self-pain vs stranger and 2) self-pain vs RP. Higher-level analyses ($z > 3.1$, $p < .05$) averaged these contrasts and empathy ratings were entered as a regressor to identify neural correlates of empathy for RP and stranger, respectively.

Empathy ratings were higher for the RP ($p = .06$; $CI_{95} = .05, 1.8$) than the stranger, despite no significant ($p = .60$) differences in reported pain between the stranger and RP. When compared to self-pain, viewing the stranger and RP during painful heat was associated with higher CBF in the posterior cingulate cortex, a central node of the self-referential default mode network. Importantly, higher empathy for the romantic partner was associated with higher CBF in the bilateral amygdala ($r = .54$, $p = .04$). There were no significant neural correlates of empathy for the stranger.

These preliminary findings provide support, using perfusion fMRI, that higher amygdala activation is associated with higher empathy, a mechanism supporting higher affective empathy and social contagion.

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Poster

630. Neuroimaging of Pain in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 630.10

Topic: D.02. Somatosensation – Pain

Support: NIDA R01 DA046064
NIBIB R01 EB026549

Title: Data harmonization improves quantitative out-of-study fMRI BOLD signal decoding with MVPA

Authors: *B. PETRE[1], M. A. LINDQUIST[3], T. D. WAGER[2];
[2]Psychological and Brain Sci., [1]Dartmouth Col., Hanover, NH; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: BOLD contrast amplitude differs across sites and studies due to equipment, acquisition parameters and statistical designs variability, while intrinsic physiological confounds like baseline blood oxygenation impede generalization across subjects. The increase in multi-site and multi-study analysis has created a pressing need to address such concerns. Here we evaluate 12 data driven harmonization methods that allow for control for these factors in multivariate pattern analysis of contrast maps from 9 previously collected datasets (306 participants).
All studies involved noxious thermal stimuli. We tested the ability to predict stimulus temperature out-of-study from harmonized and unharmonized GLM contrast images using partial least squares. The twelve harmonization methods included 4 methods of rescaling individual contrast images, and "batch" methods, including ComBat, that rescaled images jointly either treating subjects or studies as batches (4 methods each). A quasi-cross validation approach was used: models were trained on one study to predict outcomes in the remaining 8 studies, simulating a study validation effort. Mean squared error (MSE) of predicted stimulus intensity quantified model performances. We further subdivided MSE into independent variance bias, between study error, between subject error, and within subject error terms.
Except for ComBat, all methods significantly improved MSE in out-of-study predictions relative to unharmonized data (exact Friedman rank sum difference (FRSD) test). Most methods improved bias, between study error and within study error ($p < 0.05$, FRSD test), especially individual contrast image based harmonization, and no harmonization method showed a significant increase in MSE or any of its subcomponents. However, no method showed significant rank order improvements in between subject error.
We demonstrate greater ability to control for differences between studies than between subjects, but several methods show constructive amplitude correction within subjects as well. This indicates an ability to equate relative differences across subjects. Contrast image z-scoring in particular offers a computationally efficient, versatile and effective method of BOLD harmonization for out-of-subject and out-of-study prediction, while more sophisticated methods like ComBat may be more suitable when experimental designs are deliberately matched across studies or subjects. Our results indicate the extent to which amplitude information may be

informative in BOLD data and provide reference guidelines for generalizing results of multivariate pattern analysis across subjects and studies.

Disclosures: B. Petre: None. **M.A. Lindquist:** None. **T.D. Wager:** None.

Poster

631. Cannabinoids, NMDA Receptors and Pain

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 631.01

Topic: D.02. Somatosensation – Pain

Support: DoD W81XWH-18-1-0746
DoD, W81XWH-21-1-0544

Title: Targeting T-type calcium channels and the endocannabinoid system to treat SCI-induced neuropathic pain.

Authors: *E. SIPPLE[1], H. LIU[2], K. GUNARATNA[2], J. LAUZADIS[2], M. KACZOCHA[2], M. PUOPOLO[2];
[1]Anesthesiol., Med. Scientist Training Program, Stony Brook, NY; [2]Anesthesiol., Stony Brook Med., Stony Brook, NY

Abstract: Chronic neuropathic pain affects up to 60-70% of people leaving with spinal cord injury (SCI). Recent work from our laboratory showed that the increased activity of T-type calcium channels induced by the injury is responsible for driving nociceptors' hyperexcitability and for promoting the development/maintenance of SCI-induced neuropathic pain (SCI-pain) (Lauzadis et al., J Neurosci. 2020 Sep 16;40(38):7229-7240). Previous reports have shown that endocannabinoids inhibit calcium channels, raising the possibility that inhibition of T-type calcium channels in nociceptors by 2-arachidonoylglycerol (2-AG) and/or anandamide (AEA) may reduce nociceptors' hyperexcitability and SCI-pain. The goal of this work is to test the hypothesis that inhibition of endocannabinoid catabolizing enzymes *monoacylglycerol* lipase (MAGL) and fatty acid amide hydrolase (FAAH), with subsequent elevation of 2-*AG* and AEA levels, respectively, will reduce the activity of T-type calcium channels in nociceptors and rescue SCI-pain. SD rats (300-350 g) were used in this study. SCI was performed by a midline spinal cord contusion at T10 by using an Infinite Horizon Impactor (150 kilodynes, 1s dwelling time). The mechanical allodynia was assessed with the von Frey filaments by using the up-down method with the 50% threshold. The conditioned place preference (CPP) paradigm was used to assess the spontaneous pain. The action potential clamp technique was used in dissociated dorsal root ganglia (DRG) neurons isolated from SCI and sham rats to measure the interspike T-type calcium charge (sensitive to 1 μM TTA-P2) from the afterhyperpolarization of the first action potential to -50 mV before the second action potential. The 50% mechanical threshold dropped from 19.5±8.2 g (pre-injury) to 13.6±5.6 g (post-SCI). Vehicle injection changed the 50% mechanical threshold to 15.2±3.5 g. MJN110 (10 mg/kg, MAGL inhibitor) increased the 50%

mechanical threshold to 37.7±10.7 g at 1-hour post-injection and to 21.4±4.7 g (n=6) at 3-hour post injection. PF3845 (10 mg/kg, FAAH inhibitor) increased the 50% mechanical threshold from 13.6±5.6 g (post-SCI) to 27.7±4.7 g at 1-hour post-injection and to 27.0±4.8 g at 3-hour post-injection. In voltage clamp experiments in nociceptors isolated from the same cohort of rats the interspike T-type calcium charge dropped from 75±14 pC/pF (n=18) in control to 39±13 pC/pF (n=14) in the presence of 5 µM MJN110 and to 9±2 pC/pF (n=12) in the presence of 5 µM PF3845. Taken together, our data suggest that inhibition of endocannabinoid catabolizing enzymes MAGL and/or FAAH reduces the activity of T-type calcium channels in SCI-nociceptors and rescues SCI-pain.

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Poster

631. Cannabinoids, NMDA Receptors and Pain

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 631.02

Topic: D.02. Somatosensation – Pain

Title: Inhibition of DAGLα and DAGLβ in the periphery reduces pain behaviors through selective suppression of nociceptor-like activity.

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Abstract: Diacylglycerol lipase (DAGL) alpha and beta (DAGLα and DAGLβ) are enzymes that hydrolyze diacycglycerol lipids, to produce monoacylglycerols, including 2-arachidonoylglycerol (2-AG), a well-established endocannabinoid signaling ligand. In a subset of tissues, including those of the immune and nervous system, 2-AG serves as a major source of arachidonic acid, a precursor for pro-inflammatory and pro-nociceptive eicosanoid lipids. Genetic deletion or chemical inhibition of DAGLβ has demonstrated anti-nociceptive effects in neuropathic and inflammatory rodent models of pain (Wilkerson et al. 2016). Here, we show that a potent and selective dual DAGLα/β inhibitor is peripherally restricted and robustly suppresses 2-AG concentrations. Behavioral investigation in rodent models of inflammatory and neuropathic pain reveal that pharmacological inhibition of peripheral DAGLα/β produces analgesic-like effects. To shed light on the neurophysiological underpinnings of the observed behavior, we developed an in vivo extracellular single-unit recording assay in the dorsal horn spinal-cord to interrogate mechanically evoked activity in an intact sensory circuit. We observed suppression of wide-dynamic range (WDR) and nociceptor-like single-units upon graded mechanical stimulation after intravenous dosing of our DAGLα/β inhibitor. These data support a potential role of DAGL in controlling production of proinflammatory eicosanoids that modulate

sensory information from the periphery to the spinal cord which is achieved using a novel compound that is specific, restricted and potent for targeting DAGLα/β.

Disclosures: **Y. Tufail:** None. **D. Herbst:** None. **J. Ha:** None. **C. Hutton:** None. **C. Henry:** None. **T. Andalis:** None. **J. Blankman:** None. **C. Guijas Mate:** None. **J. Moody:** None. **J. Wiener:** None. **D. Kummer:** None. **K.B. Simonsen:** None. **J.R. Clapper:** None.

Poster

631. Cannabinoids, NMDA Receptors and Pain

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 631.03

Topic: D.02. Somatosensation – Pain

Support: Irish Research Council. Laureate Award (IRCLA/2017/78)

Title: Effects of intra-dorsolateral periaqueductal grey administration of CB_1, CB_2, and PPARα agonists on nociceptive behaviour following peripheral nerve injury in male and female rats

Authors: *L. BOULLON[1,2,3], M. I. FERDOUSI[1,2,3], D. P. FINN[1,2,3], Á. LLORENTE-BERZAL[1,2,3];
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Abstract: Activation of cannabinoid CB_1 and CB_2 receptors in the descending pain modulatory pathway suppresses nociceptive signalling. Systemic administration of an inhibitor of the endocannabinoid degradation enzyme fatty acid amide hydrolase (FAAH) produces sex-dependent antinociceptive effects in a rat model of neuropathic pain, with concomitant alterations in endocannabinoid levels in the periaqueductal grey (PAG) (Boullon et al., 2021). In the present study, the selective cannabinoid receptor agonists ACEA (CB_1, 0.5pmol/side) and JWH133 (CB_2, 3nmol/side), and the selective peroxisome proliferator-activated receptor alpha (PPARα) agonist GW7647 (7.96nmol/side) were administered bilaterally to the dorsolateral sub-columns of PAG (dlPAG) of male and female rats to examine potential sex differences in the effects of these drugs on nociceptive behaviour following peripheral nerve injury. Von Frey and acetone drop tests assessed alterations in mechanical and cold hypersensitivity following acute intra-dlPAG administration (200nL/side) of ACEA, JWH133, GW7647 or vehicle (100% DMSO) in male and female adult (8-9 weeks old) Sprague-Dawley rats (n=5-8/group), 17 days post Spared Nerve Injury (SNI). Data were analysed, where appropriate, using two-way ANOVA with *post hoc* Tukey's test or non-parametric equivalent Kruskal Wallis test followed by *post hoc* Mann Whitney-U test with Bonferroni-Holm correction. $P< 0.05$ was considered significant. Intra-dlPAG administration of JWH133 or GW7647 (but not ACEA) significantly reduced ($p<0.01$) mechanical hypersensitivity in female-SNI rats, with similar trends that did not reach statistical significance in male-SNI, rats. In the acetone drop test, cold hypersensitivity did not differ between the experimental groups at the time point tested. Our data suggest that activation

of CB_2 receptors and PPARα in the dlPAG may reduce SNI-induced mechanical hypersensitivity more robustly in female rats than in male rats, proposing potential sex differences in CB_2- and PPARα-associated mechanisms.
References: Boullon L, Crudden S, Finn DP, Llorente-Berzal A. Sex-dependent antinociceptive effects of fatty acid amide hydrolase but not monoacyl glycerol lipase inhibition following peripheral nerve injury in rats. European Neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology 53(8-9):S213-S214. . December 2021. DOI: 10.1016/j.euroneuro.2021.10.280

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Poster

631. Cannabinoids, NMDA Receptors and Pain

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 631.04

Topic: D.02. Somatosensation – Pain

Support: National Research Foundation of Ukraine Grant 2020.01/0266

Title: Cannabidiol reduces nociceptive response in acute respiratory distress syndrome.

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Abstract: This study aimed to research how chronic airway inflammation and spontaneous pulmonary afferents activity may be altered by the application of CB_1/CB_2 receptor antagonist cannabidiol (CBD) and CB_1 receptor inverse agonist rimonabant. In addition, to investigate the involvement of the nervous system in the regulation of the immune response. The anesthetized rats were artificially ventilated with tidal volume (TV) and respiratory frequency was set at 10 ml/kg and 60 breaths/ min (normal ventilation). After a midline laparotomy, both vagal nerve trunks were cut just below the diaphragm to eliminate afferent signals arising from lower visceral organs. Primary afferents were characterized and subdivided into “regular”(mechanosensitive A-fibers) and “irregular” (nociceptive C-fibers) according to their activity in the ventilatory cycle. We used the rat’s ARDS model induced by prolonged artificial pulmonary hyperventilation (TV 30 mL/kg, at 60 breaths/min for 4 hours) with poly(I:C) 15 mg/kg. All animals were randomly divided into four groups and tested according to the same experimental protocol (hyperventilation with poly(I:C) for four hours). Group I: control group. Group II: CBD 100 μg/kg was injected in a venous catheter near the right atrium. Group III: CBD 100 mg/kg was

injected intraperitoneally. Group IV: rimonabant 1 mg/kg was injected in a venous catheter near the right atrium. All rats were sacrificed after the experiment and the lungs were lavaged and lung tissue was preserved for immune and histology study. In the control group continued pulmonary hyperventilation with high TV gradually increased the spike frequency by 161 % compared to the control value. However, pulmonary fiber impulse activity did not return to the control value after returning to normal ventilation parameters at the end of the experiment ($P < 0.05$). Injection of cannabinoid receptor antagonist rimonabant (1 mg/kg) had no significant effect on fiber activity compared to the control group. CBD had a concentration-dependent inhibitory effect on afferent nerve activity in response to injury induced by prolonged artificial pulmonary hyperventilation. CBD at doses of 100 μg/kg and 100 mg/kg decreased spike frequency by 21.5 and 54.6 %, respectively, during hyperventilation ($P < 0.05$). And, nerve activity returned to the control value after returning to normal ventilation parameters at the end of the experiment. This assay allowed the description of an inhibitory effect of CBD on afferent nerve activity in response to lung injury, suggesting CBD could alleviate pulmonary nociception.

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Poster

631. Cannabinoids, NMDA Receptors and Pain

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 631.05

Topic: D.02. Somatosensation – Pain

Support: R01AT011517

Title: Terpenes from Cannabis sativa are Anti-Nociceptive in a Lipopolysaccharide (LPS)-Induced Inflammation Model in Mice

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Abstract: *Cannabis sativa* contains moderate to high levels of the terpenes α-Humulene, β-Caryophyllene, β-Pinene, Geraniol and Linalool. Our lab has shown that administration of these isolated terpenes produces an anti-nociceptive effect in both acute and chronic pain models. We sought to determine if these terpenes could reduce inflammatory pain, and if global terpene administration influences local cytokine production during inflammation. We induced inflammation in male and female CD-1 mice via lipopolysaccharide (LPS) injection into the left hind paw (100ng). The isolated terpenes, or vehicle control, were administered via intraperitoneal (IP) injection (200mg/kg) and mechanical allodynia was measured using Von Frey filaments on the hind left paw. The terpenes α-Humulene, β-Caryophyllene and Linalool

produced robust antinociception in the LPS inflammatory model. The mechanical allodynia of animals treated with β-Pinene or Geraniol was not significantly different from vehicle control animals, suggesting they may be less effective for reducing inflammatory pain. Right (contralateral control) and left (ipsilateral) hind paw tissue samples were collected for cytokine analysis. RNA was extracted from the paw samples using Trizol and converted to cDNA. RT-qPCR was used to measure the relative RNA in each sample for each cytokine (IL-6, IL-10, and TNFα). Increased levels of IL-6 and IL-10 were found in the LPS injected paw of animals treated with Geraniol or α-Humulene, in comparison to vehicle treated animals. β-Pinene treatment increased IL-10 mRNA. TNFα levels did not differ between treatment and vehicle groups for any terpene. This preliminary data suggests some terpenes may locally increase the production of anti-inflammatory cytokines which act as negative feedback regulators to decrease the production of pro inflammatory cytokines, and could explain their efficacy in reducing inflammatory pain. Together these findings expand our work on the pharmacological properties of terpene compounds, and suggests that terpenes could be alternate non-opioid, non-cannabinoid therapies for the treatment of inflammatory pain.
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Disclosures: A.M. Schwarz: None. **T. Appel:** None. **J.M. Streicher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Botanical Results, LLC.

Poster

631. Cannabinoids, NMDA Receptors and Pain

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Program #/Poster #: 631.06

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01 AT010773

Title: Behavioral and analgesic effects of minor phytocannabinoids

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Abstract: Cannabis is increasingly used for its analgesic effects, which may be caused by one or more phytocannabinoid compounds produced by the plant. The endocannabinoid system modulates many homeostatic processes, including pain and immune function, thus offering targets for novel, non-opioid pain medicines. The goal of the present study was to determine the analgesic efficacy of four lesser-studied, minor phytocannabinoids. To measure the acute effects of each test compound, adult male and female C57BL/6J mice were treated (i.p.) with

cumulative doses of delta-8-tetrahydrocannabinol (Δ^8-THC; 6.25-100 mg/kg), cannabinol (CBN; 6.25-150 mg/kg), cannabichromene (CBC; 10-100 mg/kg), cannabicyclol (CBL; 10-100mg/kg), or vehicle and tested repeatedly in the tetrad battery (i.e., catalepsy, tail immersion, core body temp, spontaneous locomotion). A non-selective synthetic cannabinoid (WIN 55,212-2; 1-30 mg/kg, i.p.) and the major phytocannabinoid delta-9-tetrahydrocannabinol (Δ^9-THC; 1-50 mg/kg, i.p.) were included as positive controls. Mice treated with Δ^8-THC (≥12.5 mg/kg) or CBN (≥25 mg/kg) displayed classic cannabinoid effects, including acute antinociception, at slightly higher doses than Δ^9-THC (≥10 mg/kg) or WIN 55,212-2 (≥3 mg/kg). CBL induced hypothermia at high doses (≥50 mg/kg), whereas CBC had no effect. A separate group of mice were subjected to chronic constriction injury (CCI) wherein the right sciatic nerve is ligated with a silk suture to model chronic neuropathic pain. CCI causes mechanical and cold allodynia, or sensitivity to normally non-noxious stimuli, which are quantified using the von Frey and acetone tests, respectively. Mice treated with CBN (≥50 mg/kg) displayed increased mechanical allodynia, although neither CBC nor CBL (100 mg/kg) attenuated CCI-induced allodynia. Together, these findings suggest that minor phytocannabinoid compounds are psychoactive and have acute antinociceptive properties but may be less effective at attenuating chronic neuropathic pain.

Disclosures: S.O. Vanegas: None. **S.G. Kinsey:** None.

Poster

631. Cannabinoids, NMDA Receptors and Pain

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Program #/Poster #: 631.07

Topic: D.02. Somatosensation – Pain

Support: NIH Grant F31AA028445

Title: Sex differences in alcohol-induced anti-nociception and corticolimbic endocannabinoid neuroadaptations

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Abstract: Although chronic pain affects over 100 million Americans and significantly contributes to both the development and maintenance of alcohol use disorder (AUD), there is an alarming gap in knowledge regarding the mechanisms underlying the anti-nociceptive effects of alcohol. The goals of the current project were to: 1) determine the longitudinal effects of acute alcohol on persistent inflammatory pain; 2) investigate the effects of persistent inflammatory pain chronification on alcohol clearance; and 3) identify corticolimbic neuroadaptations in endocannabinoid (eCB) system signaling associated with alcohol-induced anti-nociception. Utilizing the complete Freund's adjuvant (CFA) model of inflammatory pain and intraperitoneal ethanol injection in adult female and male Wistar rats, we quantified three pain-like behaviors

(mechanical nociception, thermal nociception, pain avoidance-like behavior), serial blood ethanol levels, and eCB system protein levels. Using nociceptive behaviors, alcohol produced dose-dependent analgesia in females but only modest anti-hyperalgesia in males. However, alcohol has highly efficacious at reducing pain avoidance-like behavior in males. In both cases, these pain-like behaviors were most effectively attenuated by alcohol 1 week post-CFA. Analysis of serial blood ethanol concentrations revealed that neither the presence or chronification of CFA altered alcohol clearance. Western blot analysis identified acute alcohol-induced decreases levels of the eCB synthetic enzyme diacylglycerol lipase-α (DAGLα) in female frontocortical regions (cingulate, insular, and dorsomedial prefrontal cortices), but CFA-induced increases male cingulate cortex levels of cannabinoid receptor 1 (CB1R) and the catabolic enzyme monoacylgycerol lipase (MAGL), suggesting that alcohol decreases frontocortical eCB synthesis in females while CFA decreases cingulate cortical eCB tone in males. These findings suggest that sex-specific alterations in corticolimbic eCB system protein levels may underlie the sex-dependent behavioral effects of acute alcohol. Future and ongoing studies seek to fully elucidate the role of central eCB signaling in alcohol-induced anti-nociception via MAGL inhibition and CB1R antagonism challenge. These findings will help elucidate the mechanism of analgesic action of alcohol in the context of chronic inflammatory pain states across sexes with the goal of identifying the eCB system as a novel target for treating pain in AUD patients.

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Poster

631. Cannabinoids, NMDA Receptors and Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS111976
NIH Grant NS115441

Title: The role of an extracellular kinase in NMDAR-dependent pain

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Abstract: EphB/ephrinB signaling plays a key role in synaptic function and is linked to several neuropathological conditions, including neuropathic pain. The EphB2 receptor tyrosine kinase is known to interact with N-Methyl-D-aspartate glutamate receptors (NMDAR), resulting in increased surface retention of NMDARs at the synapse and an enhancement of postsynaptic currents. As changes in NMDAR signaling have been heavily implicated in the development of both acute and chronic pain, targeting this receptor interaction may produce clinically relevant therapeutics. We have recently demonstrated that the phosphorylation of a tyrosine residue (Y504) on the extracellular domain of the EphB2 receptor is necessary and sufficient to induce its interaction with NMDARs and cause mechanical pain hypersensitivity. The kinase responsible for this phosphorylation is unknown. We hypothesized that Vertebrate Lonesome Kinase (VLK) may be the key effector. Since the phosphorylation site of interest is located on the extracellular face of the EphB2 receptor, a secreted tyrosine kinase like VLK is an ideal candidate. Our results provide strong support for our hypothesis that VLK drives pain via extracellular phosphorylation of EphBs. We show that intrathecal injection of VLK resulted in long-lasting mechanical hypersensitivity and spontaneous pain in mice. Importantly, these effects were dependent upon the NMDAR because they were blocked by AP-5. We also show evidence of endogenous VLK expression in dorsal root ganglion and spinal cord neurons of both mice and humans, and characterize the dependence of painful phenotypes on VLK expression in these tissues. Our results provide evidence for the involvement of VLK in pain behaviors and spinal cord NMDAR signaling, and identify a unique extracellular kinase as a potential target for the alleviation of pain

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Poster

631. Cannabinoids, NMDA Receptors and Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS NS111976
NIH/NINDS NS110385

Title: The role of Extracellular Tyrosine Kinase VLK in synaptic recruitment of NMDAR

Authors: *P. CHANDER[1], H. R. WASHBURN[2], D. KOLLURU[1], T. J. PRICE[3], M. B. DALVA[1];
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Abstract: NMDAR synaptic localization at cortical spine synapse is governed by the EphB family of receptor tyrosine kinases. EphB2 controls NMDAR synaptic localization and function

via a direct interaction between the extracellular fibronectin-type III domain on EphB2 and the N-terminal domain of GluN1. This direct interaction is mediated by a seemingly novel mechanism - the phosphorylation of a specific, and highly conserved tyrosine residue in Y504 on EphB2. While protein function modification by intracellular phosphorylation is one of the more widely appreciated mechanisms mediating protein-protein interactions, we propose that extracellular phosphorylation of proteins may play a similar role. A key question to be addressed is whether there are specific kinases that mediate these events. Extracellular phosphorylation is mediated by twelve extracellular kinases, six directed at serine/threonine and six directed at tyrosine. These kinases are linked to a diverse set of diseases including those related to bone development, autism spectrum disorder, and other diseases of brain dysfunction. Because the EphB-NMDAR interaction requires tyrosine phosphorylation, we focused on examining the role of the six tyrosine kinases.Our previous work showed that phosphorylation of Y504 occurs on the cell surface, therefore we tested whether any of the six kinases might be able to induce the EphB-NMDAR interaction when added exogenously to cells. When transfected into HEK293T cells or applied to HEK293T cells or neurons, only exogenous VLK/PKDCC induced EphB-NMDAR. Kinase dead VLK mutant protein failed to induce the interaction. Moreover, knockdown of VLK expression blocked the EphB-NMDAR interaction. These data indicate that VLK is necessary and sufficient for the EphB-NMDAR interaction. We next asked where VLK was localized in the brain. Synaptosome fractionation revealed that VLK is enriched in the synaptic vesicle fraction. In vitro, VLK co-transports in axons with Synaptophysin 1 (SYP1) and co-localizes with SYP1 and PSD-95 in axons, suggesting that VLK is found at presynaptic sites. These data suggest that VLK might be secreted from neurons using a mechanism similar to synaptic release. To begin to test this we asked if ephrin-B2 activation of EphB2 might induce VLK release from neurons. Ephrin-B induced VLK secretion from cortical neurons. Next we asked whether inhibition of SNARE dependent release might inhibit VLK secretion. Remarkably, pre-treatment of neurons with Botulinum toxin A blocked VLK secretion. These data suggest that VLK release is regulated by EphB signaling and VLK is necessary and sufficient to mediate EphB-NMDAR interaction.

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Poster

631. Cannabinoids, NMDA Receptors and Pain

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Program #/Poster #: 631.10

Topic: D.02. Somatosensation – Pain
NIH/NINDS NS110385
NIH/NINDS NS 111976

Title: Ephrin dependent transcriptional regulation of extracelluar tyrosine kinase vlk

Authors: D. KOLLURU[1], P. CHANDER[1], H. WASHBURN[2], T. J. PRICE[3], M. B. DALVA[1]; [1]Thomas Jefferson Univ., Philadelphia, PA; [2]Mol. Biol., Princeton Univ., Princeton, NJ; [3]Sch. of Behavioral and Brain Sci., Univ. of Texas At Dallas Neurosci. Undergraduate Program, Richardson, TX

Abstract: The Eph kinases and their ephrin partners comprise a large family of signaling molecules involved in neural plasticity, development, and disease. EphB regulate plasticity via a direct extracellular interaction with the NMDAR. The direct interaction between the extracellular domain of EphB**2** and the GluN1 subunit of the **NMDAR** is mediated by a novel extracellular phosphorylation of a single conserved tyrosine residue **(Y504)** on EphB2. Extracellular phosphorylation is an underappreciated mechanism but has been found to occur on over 2000 proteins, including many synaptic proteins. We have focused on one kinase, Vertebrate Lonesome Kinase (VLK/PKDCC) which is a secreted tyrosine directed kinase and appears to be localized at synapses. In this abstract we examine whether EphB2 signaling, and neuronal activity can regulate VLK expression. Synapses form in the first postnatal/in vivo week and mature during the following two to three weeks. Therefore, we first determined the developmental time course of VLK in rat neurons and mouse brain and found VLK expression decreased with age. We next asked whether activation of EphB2 signaling with ephrin-B treatment might induce VLK expression. Interestingly, although the level of VLK constitutive expression decreased with age, ephrin-B treatment increased the expression of VLK at only at older ages when baseline expression was lower (DIV 14, 21). VLK expression was also increased by increasing synaptic activity. Next, we asked whether the ephrin-dependent increase in VLK gene expression was NMDAR-dependent. The neurons were treated with ephrin-B in the presence of NMDAR blockers. Interestingly, blocking NMDAR activity only blocked VLK induction at DIV21, and failed to at DIV 14. These data suggest that VLK expression is dependent only on EphB signaling at DIV14 but requires neuronal activity at DIV21. To begin to understand the mechanism of VLK regulation we examined the known transcription binding patterns in the VLK promoter and found evidence for STAT3 binding. Consistent with a role for STAT3, inhibition of STAT signaling decreased in VLK expression. Thus, VLK expression is differentially regulated during synaptic development. These results provide a new avenue to explore the transcriptional regulatory role of Eph/ephrin signaling, and a better molecular understanding could provide important therapeutic insights in many brain disorders.

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Poster

631. Cannabinoids, NMDA Receptors and Pain

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Program #/Poster #: 631.11

Topic: D.02. Somatosensation – Pain

Support: NIH grant NS101880

Title: The NMDA receptor-mediated primary afferent input to spinal excitatory neurons differentially controls pain hypersensitivity caused by chemotherapy and nerve injury

Authors: *Y. HUANG, H. CHEN, D. JIN, S.-R. CHEN, H.-L. PAN;
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Abstract: Increased NMDA receptor (NMDAR) activity in the spinal dorsal horn is a hallmark of chronic neuropathic pain. The spinal cord dorsal horn contains excitatory interneurons expressing vesicular glutamate transporter-2 (VGluT2) and inhibitory interneurons expressing vesicular GABA transporter (VGAT), which have opposing roles in processing nociceptive input. However, it is unclear how NMDARs regulate primary afferent input to excitatory and inhibitory neurons in the spinal cord in neuropathic pain. In this study, we showed that treatment with a chemotherapy drug, paclitaxel, and spared nerve injury (SNI) in mice both caused a large increase in the frequency of miniature excitatory synaptic currents (EPSCs) and the amplitude of EPSCs evoked from the dorsal root in VGluT2-expressing dorsal horn neurons, which were reversed by the NMDAR antagonist AP5. In contrast, neither paclitaxel treatment nor SNI had significant effect on miniature EPSCs or evoked EPSCs in VGAT-expressing dorsal horn neurons. In mice with conditional GluN1 knockout in primary sensory neurons (Grin-cKO mice; produced by crossing Grin-floxed and Advillin-Cre mice), treatment with paclitaxel failed to induce tactile allodynia and mechanical and heat hyperalgesia. Strikingly, SNI still caused a profound and long-lasting pain hypersensitivity in Grin-cKO mice and in tamoxifen-induced Grin cKO from primary sensory neurons in adult mice. Furthermore, SNI caused a similar increase in the activity of postsynaptic NMDARs in VGluT2-expressing dorsal horn neurons and a depolarizing shift in GABA reversal potential in wild-type and Grin-cKO mice. In addition, knockdown of GluN1 in the spinal cord and DRG neurons via intrathecal injection of AAV-Cre vectors in Grin1-floxed mouse completely blocked pain hypersensitivity induced by SNI. These findings suggest that presynaptic NMDARs predominantly potentiate primary afferent input to spinal excitatory neurons in neuropathic pain conditions. Although presynaptic NMDARs are essential for chemotherapy-induced neuropathic pain, postsynaptic NMDARs in spinal dorsal horn neurons play a key role in the development of neuropathic pain after traumatic nerve injury. Our study reveals distinct functional significance of presynaptic and postsynaptic NMDARs in regulating cell type-specific nociceptive input in the spinal dorsal horn and in neuropathic pain development with different etiologies.

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Poster

631. Cannabinoids, NMDA Receptors and Pain

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Program #/Poster #: 631.12

Topic: D.02. Somatosensation – Pain

Title: Protective effect of Dimethyl Fumarate (DMF) in post-operative pain

Authors: *A. ARDIZZONE, G. CASILI, M. LANZA, A. FILIPPONE, L. CUCINOTTA, A. CAPRA, M. CAMPOLO, I. PATERNITI, S. CUZZOCREA, E. ESPOSITO;
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Abstract: Protective effect of Dimethyl fumarate (DMF) in post-operative painAlessio Ardizzone, Giovanna Casili, Marika Lanza, Alessia Filippone, Laura Cucinotta, Anna Paola Capra, Michela Campolo, Irene Paterniti, Salvatore Cuzzocrea and Emanuela Esposito Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina
AbstractBackground. Pain should be considered one of these undesirable events related to perioperative phase, therefore optimal pain management is mandatory for early rehabilitation after surgery. Actually, the management of post postoperative (PO) pain has generally been shown to be inadequate, therefore acquiring novel acknowledgment on PO pain mechanism would increase the therapeutic options available. There is accumulating evidence to implicate N-methyl-d-aspartate (NMDA) receptors to the induction and maintenance of central sensitization during pain states. Therefore, NMDA receptor antagonists have been implicated in perioperative pain management. Recent advances demonstrated that Dimethyl fumarate (DMF), a non-opioid and orally bioavailable drug, is able to resolve neuroinflammation through mechanisms that drive nociceptive hypersensitivity. Therefore, in this paper, we evaluated the role of DMF on pain and neuroinflammation in a mouse animal model of PO pain.**Methods.** An incision of the hind paw was performed and DMF, at two different doses (30 and 100 mg/kg) was orally administered for five days. Mechanical allodynia, thermal hyperalgesia, and motor dysfunction were assessed daily for five days after surgery. Mice were sacrificed at day 7 after PO pain induction and hind paw and lumbar spinal cord tissues were harvested for histological and molecular analysis. **Results**. DMF administration significantly reduced hyperalgesia and allodynia, alleviating motor disfunction. Treatment with DMF significantly reduced histological damage, counteracted mast cell activation and reduced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)
inflammatory pathway, also downregulating tumor necrosis factor-α (TNF-α), Interleukin-1β (Il-1β) and Il-4 expression. Interestingly, DMF treatment reduced the activation of the NMDA receptor subtypes NR2Band NR1 and the NMDA receptor-interacting PDZ proteins including PSD-93 e PSD-95. Furthermore, DMF interfered with calcium ion function, modulating nociception.**Conclusions**. Thus, DMF administration modulated PO pain, managing NMDA signaling pathways. The results suggest that DMF positively modulated persistent nociception related to PO pain, through predominantly NMDA receptor-operated calcium channels.

Disclosures: **A. Ardizzone:** None. **G. Casili:** None. **M. Lanza:** None. **A. Filippone:** None. **L. Cucinotta:** None. **A. Capra:** None. **M. Campolo:** None. **I. Paterniti:** None. **S. Cuzzocrea:** None. **E. Esposito:** None.

Poster

631. Cannabinoids, NMDA Receptors and Pain

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 631.13

Topic: D.02. Somatosensation – Pain

Support: Grants-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (No. 19147263)

Title: Effects of magnesium sulfate pretreatment on chronic postsurgical pain in rats

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Abstract: Chronic postsurgical pain (CPSP) is a serious issue for many postoperative patients. Though there are numerous treatment options for the prevention of CPSP, none of them is optimal as the mechanisms of the transition from acute to chronic postoperative pain have not been elucidated. Ketamine and opioids have been administered for chronic postoperative pain treatment but induce severe adverse reactions and/or physical dependency.Here, we examined whether pre-administration of the nonselective N-methyl-D-aspartate (NMDA) receptor antagonist magnesium sulfate attenuates CPSP behavior and alters the expression of glutamate ionotropic receptor NMDA type subunit 1a (Grin1 mRNA) in a rat skin/muscle incision and retraction (SMIR) model. We assessed the effects of a single subcutaneous magnesium sulfate injection on nociceptive behaviors including guarding pain, mechanical hyperalgesia, and heat hypersensitivity in rats after SMIR surgery. We used reverse transcription-quantitative PCR (RT-qPCR) to evaluate Grin1 mRNA expression in the dorsal horn of the spinal cord on postoperative day 14. Compared with the vehicle, magnesium sulfate administration before SMIR surgery reduced mechanical hyperalgesia for 17 d Grin1 gene expression was significantly higher on the ipsilateral side than the contralateral side ($P < 0.001$) on postoperative day 14. The magnesium sulfate injection prevented Grin1 mRNA upregulation in the spinal cord dorsal horn. A single magnesium sulfate injection mitigated SMIR-induced mechanical hyperalgesia possibly by modulating Grin1 expression. Preoperative magnesium sulfate administration could prove to be a simple and safe CPSP treatment.

Disclosures: K. Kido: None.

Poster

631. Cannabinoids, NMDA Receptors and Pain

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Peer Reviewed Medical Research Program Award PR181740:W81XWH-19-1-0673 of the Congressionally Directed Medical Research Program of the

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University of Minnesota College of Pharmacy

Title: Novel antihyperalgesic agents, strategically substituted agmatines, effectively reduce hypersensitivity without abuse liability

Authors: *C. BARAJAS[1], C. D. PETERSON[2], K. F. KITTO[2], L. CAYE[3], G. L. WILCOX[5], C. A. FAIRBANKS[4];
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Abstract: There is an urgent unmet need for non-opioid analgesic therapeutics. Chronic activation of the NMDAR is a driving force of the neuropathology of chronic pain. The therapeutic potential of antagonism of the NMDAR has been pursued, however, off-site side-effects have hindered development. We previously demonstrated that the endogenous NMDAR antagonist, agmatine, is efficacious as an antihyperalgesic in animal models of various pain states, such as the inflammatory CFA model, and holds significant potential for development as an analgesic. To enhance the targeted bioavailability of systemically delivered agmatine, we designed agmatine analogs with substitutions (SSAs) intended to improve delivery to the spinal cord. It is crucial that all pharmacological compounds in development undergo testing to evaluate off-target side effects. Our objective was to evaluate the abuse liability of systemically delivered agmatine and four SSAs using two animal models of reward, conditioned place preference (CPP) and self-administration (SA). Female and male ICR mice were randomly separated into 6 groups, organized by active compound, and then further separated into active vs. control groups. Mice were then oriented to a CPP apparatus that contained two distinct compartments, which could be separated by a guillotine door. For 3 days, mice were trained to associate one side of the chamber with an active compound and the other with vehicle. Blinded experimenters administered s.c. injections twice daily and immediately restricted mice to one side of the apparatus, such that each animal received both active and control compounds each day, separated by 6 hours. On day 4, mice were allowed free access to both sides. Animals that received morphine, our positive control, showed a significant increase in preference for the morphine-paired chamber compared to saline control animals, as shown by one-way ANOVAs and Student's t-tests. Separately, subjects that received agmatine or any SSA exhibited no increase in preference for the active drug-paired chamber. No sex differences were found. Female and male Sprague Dawley catheterized rats were separated into experimental groups and were either able to self-administer for I.V. oxycodone or I.V. SSA3 infusions, vs. inactive control, over 12 hours. SSA3 dose was gradually increased over time. Rats with access to oxycodone showed significantly higher lever presses when compared to rats pressing for SSA3 as shown by AUC and Student’s t-test. These data show that neither agmatine nor any analog display abuse liability in these two rodent models of reward. These data support the continued development of agmatine and SSAs to address chronic pain.

Disclosures: C. Barajas: None. **C.D. Peterson:** None. **K.F. Kitto:** None. **L. Caye:** None. **G.L. Wilcox:** None. **C.A. Fairbanks:** None.

Poster

631. Cannabinoids, NMDA Receptors and Pain

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Topic: D.02. Somatosensation – Pain

Support: DOD CDMRP Grant MR141337
NIH NIDA T32 Grant 5T32DA007234-33

Title: Strategically Substituted Agmatines Inhibit NMDA-Evoked Behaviors Following Systemic Administration and Show Increased Plasma and CNS Exposure of Agmatine

Authors: *B. M. CLEMENTS[1], P. A. JONES[1], K. F. KITTO[2], C. PETERSON[2], G. L. WILCOX[3], C. A. FAIRBANKS[4];
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Abstract: Chronic pain remains a major disability, and treatment development to help patients is stagnant. As neuroplasticity plays a major role in chronic pain, NMDA antagonists have been put forth, but psychological and motor side effects due to complete antagonism limit use. Agmatine, an endogenous neuromodulator, antagonizes the NMDA receptor specifically at the GluN2B subunit and can reduce chronic pain behaviors without motor or neurological dysfunction. Development of agmatine as a clinical therapeutic, though, is difficult due to fast elimination from the plasma and limited blood-brain barrier permeability. Therefore, we have developed a series of agmatine-based prodrugs, the Strategically Substituted Agmatines (SSAs), designed to increase lipophilicity, plasma half-life, and blood-brain barrier permeability, thereby improving agmatine efficacy. We hypothesize that the SSAs will show metabolism to agmatine, increased CNS distribution, and greater antagonism of NMDA receptor-dependent behaviors in preclinical models.
To assess SSA and agmatine pharmacokinetics, three SSAs were administered IV via jugular catheter in male and female rats. SSA and agmatine content was measured in plasma via LC-MS/MS. For CNS distribution studies, SSA3 was administered IV via tail-vein injection in male and female rats, and plasma, spinal cord, and brain were collected and dissected. These tissues were heat-treated to stabilize agmatine and analyzed via LC-MS/MS. Each SSA showed increased plasma half-life. Agmatine plasma concentration was also increased, with SSA4 showing the greatest agmatine concentrations. Furthermore, SSA3 IV administration led to appearance of SSA3 in each CNS tissue studied, with high levels of agmatine accumulating in the brain and spinal cord.
To test the impact of SSAs on the NMDA receptor, we measured nociceptive behaviors and tail-flick hyperalgesia following intrathecal administration of NMDA in male and female mice. Following subcutaneous administration at doses in which agmatine had little effect on NMDA-evoked scratching and biting, SSA3 was able to significantly reduce these behaviors for 120 minutes. Furthermore, agmatine and all SSAs studied reduced NMDA-induced tail-flick hyperalgesia, although SSA3 showed a delayed response, beginning at 60 minutes.
Overall, these data show all the SSAs extend plasma half-life, increase plasma agmatine content,

and cross the BBB. This passage is highlighted by the inhibition of centrally-induced behaviors following NMDA administration. These data support our hypothesis and support the SSAs as novel agmatine-based prodrugs for the treatment of chronic pain.

Disclosures: B.M. Clements: None. **P.A. Jones:** None. **K.F. Kitto:** None. **C. Peterson:** None. **G.L. Wilcox:** None. **C.A. Fairbanks:** None.

Poster

632. Vestibular Central Physiology and Anatomy

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 632.01

Title: WITHDRAWN

Poster

632. Vestibular Central Physiology and Anatomy

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 632.02

Topic: D.05. Auditory & Vestibular Systems

Support: Washington University Center for Cellular Imaging
NIH R01 DC016413
NIH EYE030623
NIH EY029313
Sloan Research Fellowship
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Research to Prevent Blindness Career Development Award

Title: The organization of the gravity-sensing system in zebrafish

Authors: *Z. LIU[1], D. G. HILDEBRAND[3], J. L. MORGAN[2], Y. JIA[1], N. SLIMMON[1], M. W. BAGNALL[1];
[1]Neurosci., [2]DOVS, Washington Univ. in St. Louis, St. Louis, MO; [3]The Rockefeller Univ., New York, NY

Abstract: Motor circuits develop in sequence from those governing fast movements to those governing slow. Here we examine whether upstream sensory circuits are organized by similar principles. Using serial-section electron microscopy in larval zebrafish, we generated a complete map of the gravity-sensing (utricular) system spanning from the inner ear to the brainstem. We find that both sensory tuning and developmental sequence are organizing principles of vestibular

topography. Patterned rostrocaudal innervation from hair cells to afferents creates an anatomically inferred directional tuning map in the utricular ganglion, forming segregated pathways for rostral and caudal tilt. Furthermore, the mediolateral axis of the ganglion is linked to both developmental sequence and neuronal temporal dynamics. Early-born pathways carrying phasic information preferentially excite fast escape circuits, whereas later-born pathways carrying tonic signals excite slower postural and oculomotor circuits. These results demonstrate that vestibular circuits are organized by tuning direction and dynamics, aligning them with downstream motor circuits and behaviors.

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Poster

632. Vestibular Central Physiology and Anatomy

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Program #/Poster #: 632.03

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD grant R01DC016974

Title: Elucidating the role of muscarinic acetylcholine (mAChR) receptor signaling in efferent mediated responses of vestibular afferents in mammals

Authors: *A. SINHA, C. LEE, J. C. HOLT;
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Abstract: The peripheral vestibular system detects head position and movement through activation of vestibular hair cells (HCs) in semicircular canal cristae and otolithic organs. HCs transmit this information to the CNS by way of primary vestibular afferent neurons. The CNS, in turn, modulates HCs and afferents via the efferent vestibular system (EVS) and activation of primarily cholinergic signaling mechanisms. In mice, we previously demonstrated that activation of muscarinic acetylcholine receptors (mAChRs), during EVS stimulation, gives rise to a slow excitation that takes seconds to peak and tens of seconds to decay back to baseline. This slow excitation is mimicked by muscarine and ablated by the non-selective mAChR blockers scopolamine and atropine. While there are five distinct mAChRs (M1-M5), the subtypes driving EVS-mediated slow excitation remain unidentified and details on how these mAChRs alter vestibular function is not well understood. The objective of this study is to characterize which mAChR subtypes drive EVS-mediated slow excitation, and how their activation impacts vestibular physiology and behavior.
Methods: Both sexes of 6-14wk C57BL/6J and muscarinic knockout (KO) (M2/M4 and M3 mAChR KO) mice were used for these experiments. Single-unit spike recordings were obtained from regular and irregular vestibular afferents using glass microelectrodes while electrically stimulating EVS neurons and pharmacological treatment with mAChR subtype-selective drugs.

Vestibular sensory evoked potentials (VsEPs) were recorded before/after administration of the mAChR agonist oxotremorine. Additional vestibular behavioral assays including open field activity, postural sway, thermal profiles, and balance beam were measured with/without a 5-min vestibular challenge on an orbital shaker (125 RPMs). For all recordings, 4-9 mice/group were used, and standard statistical analyses was done in GraphPad prism.
Results: In C57Bl/6J mice, M3 mAChR antagonists were more potent at blocking slow excitation than M1 antagonists, while M2/M4 blockers were ineffective. While unchanged in M2/M4 KO mice, EVS-mediated slow excitation in M3KO animals was reduced or absent in irregular, but not regular, afferents. In agreement, VsEPs, proposed to originate from irregular afferents, were less enhanced by mAChR activation in M3KOs compared to controls. Finally, in M3KO mice, distinct behavioral phenotypes were observed in open field activity, postural sway, and thermal profiles, but not balance beam.
Conclusion: M3mAChRs mediate efferent mediated slow excitation in irregularly-firing mammalian vestibular afferents.

Disclosures: A. Sinha: None. **C. Lee:** None. **J.C. Holt:** None.

Poster

632. Vestibular Central Physiology and Anatomy

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Program #/Poster #: 632.04

Topic: D.05. Auditory & Vestibular Systems

Support: CIHR 162285
NIH R01-DC002390
NIH R01-DC018061

Title: Encoding of self-motion stimulus amplitude by subcortical and cortical vestibular areas: implications for perception.

Authors: *I. MACKROUS[1], J. CARRIOT[2], K. E. CULLEN[3], M. J. CHACRON[1];
[2]Physiol., [1]McGill Univ., Montreal, QC, Canada; [3]Dept. of Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD

Abstract: Natural stimuli across sensory modalities frequently consist of a fast time-varying waveform whose amplitude (i.e., envelope) varies more slowly and carries critical information for perception. Although psychophysical studies have shown that humans perceive the amplitude of self-motion stimuli accurately (Mallery et al. 2010), the underlying neural substrate remains unknown to date. To answer this question, recordings from vestibular sensitive neurons in both subcortical and cortical areas were made in response to envelope self-motion stimuli. Specifically, our dataset consisted of 25 vestibular-only (VO) neurons within the vestibular nuclei as well as 26 vestibular neurons within the parieto-insular vestibular cortex (PIVC) in awake behaving macaque monkeys. Self-motion stimuli consisted of full body rotations in the

YAW plane whose timecourse was given by a noisy waveform whose envelope varied sinusoidally at different frequencies spanning the natural range (0.05 -1 Hz). Importantly, stimulus amplitude was kept low such as not to elicit static nonlinearities (i.e., rectification, saturation) from canal afferents. Overall, we first found that VO neurons responded to envelope stimuli through changes in firing rate that tracked the detailed timecourse of the envelope with a constant gain across frequency. Surprisingly, VO firing rate responses lagged the envelope stimulus with a ~ 100 ms latency. Computational modeling revealed that envelope responses in VO neurons could not be explained by static nonlinearities (i.e., cut-off and saturation). Rather, responses could be reproduced only by including a delayed envelope signal in the model. As such, it is unlikely VO responses are simply inherited from afferents nor are extracted at the first stage of vestibular perception processing. Rather, the large latency suggests that envelope responses are generated in downstream brain areas and fed back to the vestibular nuclei. Indeed, our analysis of PIVC responses confirmed this hypothesis, as these displayed similar gains but much lower latencies (~ 22 ms). We further found that PIVC neural responses to envelope stimuli could be modeled using an inhomogeneous Poisson process. Overall, these finding challenge the notion that neurons from subcortical vestibular areas encode self-motion stimulus amplitude but rather suggest that this information is extracted in cortical areas that generate perception and fed back to subcortical areas.

Disclosures: I. Mackrous: None. **J. Carriot:** None. **K.E. Cullen:** None. **M.J. Chacron:** None.

Poster

632. Vestibular Central Physiology and Anatomy

Location: SDCC Halls B-H

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Program #/Poster #: 632.05

Topic: D.05. Auditory & Vestibular Systems

Title: The effects of passive rotations and self-generated movements on thalamic head-direction cells in virtual reality

Authors: *A. PAK, J. AARSE, J.-P. NOEL, D. E. ANGELAKI;
New York Univ., New York, NY

Abstract: Multisensory integration is crucial for animals to effectively navigate the world and hence their survival. Despite a large body of literature on multisensory processing, it is unclear how different sensory modalities dynamically interact to produce the final percept. The head direction (HD) system provides a unique opportunity to answer this question. It is widely accepted that HD cells act as a “neural compass” maintaining allocentric representation of head orientation in the environment. Their activity profile represents the output of a ring attractor that integrates velocity signal to update animal’s current orientation. This velocity signal might be influenced by vestibular, visual, olfactory cues, and efference copies of motor commands. We utilized virtual reality (VR) and Neuropixel recordings in mouse anterior thalamic nuclei to investigate how thalamic HD cells behave under three different conditions: head-fixed passive

rotation, head-fixed self-generated rotation (closed-loop platform rotation), and head-free rotation. First, we found that the ring attractor was not active during passive rotations in head-fixed mice in VR. Interestingly, changing the axis of rotation by titling the platform resulted in emergence of the ring attractor, suggesting that gravity might act as a landmark. However, minimizing the head movements in the real world using a custom-built head restrainer did not affect HD cells in mice. Second, we leveraged a new VR setup that allows a free head rotation to investigate whether active self-generated rotation vs self-generated platform rotation in a head fixed condition would differentially influence the HD system. We observed intact HD tuning and a ring attractor in a head-free condition, whereas tuning was reduced, and ring attractor activity did not properly integrate the velocity signal in head fixed condition. These results suggest that gravity might provide additional cues for the HD system and self-generated movements are not sufficient to recover ring attractor activity, but active head rotation is necessary.

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Poster

632. Vestibular Central Physiology and Anatomy

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant 2T32DC000023-36A1
NIH U01-90084554

Title: Impacts of vestibular loss on macaque gaze shifts and stabilization during locomotion

Authors: *O. R. STANLEY, R. WEI, K. E. CULLEN;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: During locomotion, stable and accurate control of gaze - the sum of the head's orientation in space and the eyes' orientation within the head - is critical for obtaining relevant visual information but is made challenging by the body's movement. The vestibular system makes critical reflexive contributions to this stability, particularly the vestibulo-ocular reflex, which keeps the fovea on-target by driving eye movements to counteract head movement, and the vestibulo-collic reflex, which helps keep the head steady when the body moves, including during locomotion. Patients with vestibular dysfunction thus face challenges in navigating activities of daily living, such as a greater risk of falls. Here, we sought to better characterize nonhuman primates as a preclinical model of vestibular loss during complex, naturalistic behavior.
Specifically, to understand the contributions of the vestibular system to gaze control during locomotion, we assessed the gaze control strategies of two normal rhesus macaques and one with long-term complete bilateral vestibular loss during walking both on a treadmill at varied speeds and during repeated passages of a linear walkway. We recorded single-eye video-oculography

using a head-mounted camera and used a head-mounted 6D inertial measurement unit and retroreflective markers to capture head movement & orientation. Animals' gait was captured in 3D via markerless feature tracking using synchronized cameras set around the behavioral apparatus.
We first asked whether there was a systematic relationship between the timing of gaze shifts and gait cycle during locomotion. Analysis of two phase-locking indices (vector strength and distribution entropy) of gaze shifts relative to gait cycle demonstrated that this was not the case; in all animals, the timing of gaze shifts was independent of gait cycle phase. We then examined the prevalence of fixation versus gaze shifts during locomotion. To identify periods of rapid gaze reorientation versus stabilization we compared eye- and head-movements. We found that vestibular loss disrupted the ability of the animal to coordinate eye and head movements to keep gaze stable, resulting in a smaller fraction of time spent with gaze fixed in space. While normal animals maintained stable gaze for between half and two thirds of a given gait cycle, the ability to coordinate eye and head movements to maintain stable gaze was disrupted in the animal with chronic vestibular loss, resulting in a smaller fraction of time spent with gaze fixed in space. We conclude that the disruption of vestibular input produces challenges that cannot be overcome by behavioral adaptation alone.

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Poster

632. Vestibular Central Physiology and Anatomy

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Topic: D.05. Auditory & Vestibular Systems

Support: CIHR 162285
NIH R01-DC002390
NIH R01-DC018061

Title: The coding strategies of neurons along ascending vestibular pathways are adapted to the statistics of natural self-motion.

Authors: *J. CARRIOT[1], I. MACKROUS[1], K. E. CULLEN[2], M. J. CHACRON[1];
[1]Physiol., McGill Univ., Montreal, QC, Canada; [2]Dept. of Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD

Abstract: Understanding the neural mechanisms by which sensory input gives rise to perception and behavior requires knowledge of how the sensory representation changes across successive brain areas. Here we investigated how natural self-motion stimuli are represented at distinct stages of processing in the vestibular pathways from subcortical vestibular nuclei to central cortical areas that contribute to both vital reflexes as well as spatial perception. We recorded the activity of single neurons within the vestibular nuclei (VN), the ventral posteriolateral (VPL)

Thalamus, and the parieto-insular vestibular cortex (PIVC), in response to both artificial and naturalistic self-motion. We first found that responses to artificial self-motion stimuli were similar across stages and consisted of changes in firing rate that followed the stimulus' timecourse. In contrast, neurons at each of these three stages demonstrate increasingly optimized encoding for naturalistic stimuli. Specifically, while VN neurons transmitted information about naturalistic self-motion through a combination of changes in firing rate and precise spike timing, VPL neurons efficiently and unambiguously represented self-motion exclusively through changes in firing rate. Surprisingly, we further found that PIVC neurons reliably signaled the occurrence of specific stimulus features via bursts of firing. In contrast, individual PIVC neurons do not provide reliable information as to the stimulus' detailed timecourse but instead fire bursts of action potentials that reliably detect rapid changes in head velocity during naturalistic. Further analysis at the population level revealed that the representation of naturalistic stimuli is distributed across PIVC neurons, as pooling their activities provided reliable information as to the stimulus' detailed timecourse. Taken together, our results show an evolution in the coding strategy used by ascending vestibular pathways from the VN to cortex to represent naturalistic self-motion. Notably, neurons at each level respond in a fundamentally different manner to naturalistic as opposed to artificial self-motion stimuli. Perhaps the most striking difference was found in PIVC neurons, which responded to artificial self-motion stimuli through changes in firing rates that followed the stimulus' detailed timecourse but instead reliably detected features of naturalistic self-motion stimuli through burst firing. This finding suggests that our perception of natural self-motion stimuli differs fundamentally from that of artificial stimuli that have been used to date and that a critical rethinking of self-motion perception may be necessary.

Disclosures: **J. Carriot:** None. **I. Mackrous:** None. **K.E. Cullen:** None. **M.J. Chacron:** None.

Poster

632. Vestibular Central Physiology and Anatomy

Location: SDCC Halls B-H

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Topic: D.05. Auditory & Vestibular Systems

Support: CIHR PJT-153257
CFI
CIHR Fellowship to FB

Title: Reference frames for encoding vestibular signals in the posterior vermis and rostral fastigial nucleus of the cerebellum across changes in head and body orientation

Authors: *F. BURON, C. Z. MARTIN, A. M. GREEN;
Univ. de Montréal, Montréal, QC, Canada

Abstract: Many daily tasks (e.g., postural control, reaching) rely on estimates of body motion with respect to specific body axes and/or gravity. To contribute to such tasks, head-centered

vestibular signals must be transformed into body- and world-centered reference frames. Previously, we showed that cell populations in the rostral fastigial nucleus (rFN) reflect the 3D spatial transformation of otolith signals required to compute estimates of body translation (Martin et al., 2018). Most rFN cells also preferentially encoded translation as compared to tilt, and while their tuning properties were distributed between head- and body-centered, they were consistent with the rFN reflecting a late stage of the head-to-body reference frame transformation. These observations suggested a potential role in this transformation for areas of the posterior cerebellar vermis (PV; lobules 9,10) that have been implicated in computing translation estimates from ambiguous otolith signals (Yakusheva et al., 2007; Laurens et al., 2013). Notably, however, we found that PV cells encoded otolith signals during translation exclusively in head-centered coordinates. Here, we extend these results to examine the reference frames in which canal-derived estimates of tilt are encoded in the PV as well as to distinguish between body- vs world-centered vestibular coding in the rFN. We recorded PV Purkinje and rFN cells in 3 rhesus monkeys for different combinations of translation and tilt motions in 3D space as well as for earth-vertical-axis rotations. Cell tuning was characterized with the head and body upright, after static reorientation of the head relative to the body and after reorientation of the body relative to gravity. PV cells reflected mainly head-centered coding of otolith signals. Furthermore, while both translation- and tilt-selective cells carried canal signals that had been spatially transformed into world/gravity-referenced estimates of tilt, such tilt estimates were also encoded in head-centered coordinates. In contrast, rFN cells reflected otolith signals that ranged from head- to body-centered (but not world-centered) and canal signals that reflected complex combinations of head, body and world/gravity-centered reference frames. Our results are thus compatible with theoretical models (e.g., Green et al., 2004) proposing that the PV combines spatially-transformed canal signals with head-centered otolith signals to compute head-centered estimates of translation and tilt. However, they also suggest that the computations to construct body-centered translation and tilt estimates may occur elsewhere, within parts of the anterior vermis (Manzoni et al., 1999).

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Poster

632. Vestibular Central Physiology and Anatomy

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Program #/Poster #: 632.09

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01 DC2390

Title: Prosthesis-evoked eye and head movements are linear beyond the maximum afferent firing rate: implications for vestibular prosthesis dynamic range

Authors: *K. P. WIBOONSAKSAKUL[1], C. C. DELLA SANTINA[2], K. E. CULLEN[1]; [1]Biomed. Engin., [2]Otolaryngology-Head and Neck Surgery, Johns Hopkins Univ., Baltimore, MD

Abstract: Bilateral vestibulopathy can lead to debilitating visual and postural instability in addition to constant dizziness. One approach to improve the quality of life in this patient population is a vestibular prosthesis that senses head rotation and transforms it into vestibular afferent stimulation, substituting for the damaged periphery. Early results from clinical trials, while encouraging, have shown only partial functional improvements. Specifically, the evoked vestibulo-ocular reflex (VOR) fails to fully stabilize the gaze (i.e., gain<1). Current strategies to improve the VOR gain have notable downsides; increasing stimulation current level can lead to non-specific activation, while modulating the stimulation rate more strongly for a given head velocity can lead to earlier saturation and narrower dynamic range. One key parameter that remains uninvestigated, however, is the maximum stimulation rate. This is typically set at the maximum endogenous afferent firing rate (~350 Hz) based on the assumption that each stimulation pulse will reliably evoke a spike in the afferents. Accordingly, here we investigated the linearity of prosthesis-evoked eye and head movements across a large stimulation frequency range. We recorded eye and head movements, in addition to neck EMG activity, in one monkey during acute afferent stimulation up to 1500 Hz. Eye movement data showed linear response up to ~600-800 Hz before starting to plateau. Head movement and neck EMG exhibited sooner saturations with linear response up to ~400 Hz. These results contradict the common assumption that each stimulation pulse will reliably evoke an action potential in the afferents (i.e., 100% stimulation efficacy). Thus, finally, we examined this efficacy assumption using a model of the VOR pathway to predict eye velocity based on stimulation rate. The model yielded accurate predictions only when stimulation efficacy was reduced to 28%, a value similar to that from afferent recordings during stimulation previously published by our group. Due to the <100% efficacy, by setting the maximum stimulation rate equal to the maximum afferent firing rate, the conventional approach artificially limits the dynamic range of the prosthesis. Taken together, our results suggest that, by accounting for the actual physiological efficacy of afferents responses, the vestibular prosthesis can better utilize the range of afferent firing rates. We suggest a complementary approach combining eye movement recording and modeling can be used to optimize the prosthesis gain and dynamic range for prosthesis users in the clinic.

Disclosures: K.P. Wiboonsaksakul: None. **C.C. Della Santina:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Labyrinth Devices, LLC. **K.E. Cullen:** None.

Poster

632. Vestibular Central Physiology and Anatomy

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 632.10

Topic: D.05. Auditory & Vestibular Systems

Support: Chair of Excellence, Normandy Council

Title: Influence of gravity on temporal cognition : a rodent approach

Authors: *E. MAROUANE, P. DENISE, G. CLÉMENT, V. BOUET, T. FRERET;
Unicaen / Inserm COMETE, Caen, France

Abstract: Temporal cognition is influenced by gravity. Indeed, during their stay in the International Space Station (ISS), astronauts display changes in the subjective perception of time, reported as a “time compression syndrome”. Since the vestibular system is the major brain sensor of linear acceleration (including gravity), its functional unloading during weightlessness might explain changes in time perception. In fact, vestibular nuclei are the first relay of integration of vestibular afferences in combination with several other sensory inputs. These nuclei send indirect projections to both the hippocampus and the striatum. This spreading vestibular network modulates various cognitive functions, which could also involve temporal cognition. However, the role of the vestibular network in time perception remains unknown so far and its modulation through gravitational changes could be responsible for the time compression syndrome previously reported. Herein, we set an innovative experimental protocol to identify the role of the vestibular system in time perception. Rodents were submitted to vestibular inputs changes through the use of an *in vivo* centrifuge. To hyperstimulate the peripheral vestibular receptors, rats were submitted to 2G hypergravity during either short or long delay (1 and 30 days). Indeed, while physiological changes are usually described after common 6 months mission within ISS, estimation of duration was recently reported to be affected even during parabolic flights. Temporal cognition was evaluated in rats using operant conditioning in touch screen devices. We recently developed two new behavioral paradigms adapted from clinical studies: a reproduction task and a temporal discrimination task. Yet, a first group of animals has been tested. In order to optimize our protocol, we have studied the effects of food restriction, essential for any operant learning. Strikingly, for the duration reproduction task, and after enhanced learning, the animals performed better when unrestricted ($p<0.05$) while an increase in food restriction increased performances on the temporal discrimination task ($p<0.05$). This highlights the importance of motivation but also impulsivity in rodent-adapted temporal tasks. After centrifugation, these rats will be tested again leading to complementary results that will be presented in the poster. Whether we manage to reproduce in rodents the observed alterations in humans, we plan to investigate through invasive approaches, the links between vestibular network and the neural substrate of time perception. Overall, these new insights will help to better manage the cognitive alterations reported in astronauts.

Disclosures: E. Marouane: None. **P. Denise:** None. **G. Clément:** None. **V. Bouet:** None. **T. Freret:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.01

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01-DC019394
NSF Grant SMA 1734892

Title: Progression of acoustic, phonemic, lexical and sentential neural features emerge for different speech listening

Authors: *I. KARUNATHILAKE[1], C. BRODBECK[2], S. BHATTASALI[1], P. RESNIK[1], J. SIMON[1];
[1]Univ. of Maryland Col. Park, Univ. of Maryland, College Park, MD; [2]Univ. of Maryland Col. Park, Univ. of Connecticut, Storrs, CT

Abstract: Understanding speech requires analyzing acoustic waveforms via intermediate abstract representations including, phonemes, words, and ultimately meaning along with other cognitive operations. While recent neurophysiological studies have reported that the brain tracks acoustic and linguistically meaningful units the impact of different kinds of speech information on brain responses is not well understood. Additionally, how these feature responses are modulated by top-down mechanisms and speech comprehension is not well understood. To address these, we recorded magnetoencephalography (MEG) data from 30 healthy younger participants while they listened to four types of continuous speech-like passages: speech-envelope modulated noise, narrated English-like non-words, word-scrambled narrative, and true narrative. Using multivariate temporal response function (mTRFs) analysis, we show that the cortical response time-locks to emergent features from acoustics to linguistic processes at the sentential level as incremental steps in the processing of speech input occur. Our results show that when the stimulus is unintelligible, the cortical response time-locks only to acoustic features, whereas for intelligible speech, the cortical response time-locks to both acoustic and linguistics features. For the case of narrated non-words, phoneme-based lexical uncertainty generates less activation than for true words, suggesting a lack of predictive coding error. Temporal analysis shows that the non-word onsets do generate smaller early responses than word onsets, but they also generate stronger late responses than word onsets suggesting different neural mechanisms associated with accessing lexico-semantic memory traces. For the scrambled word passages, we find additional responses based on context-independent word surprisal, but for true narrative, the responses are additionally driven by context-based word surprisal. The unigram word surprisal responses show strong late peaks for the scrambled word passage, consistent with an N400-like response. The results also show that most language-dependent time-locked responses are left lateralized, whereas lower-level acoustic feature responses are right lateralized or strongly bi-lateral. Taken together, our results show that brain responses to certain linguistic units are influenced by the speech content, the level of processing and speech features that could be attributed to evaluate perception and comprehension.

Disclosures: I. Karunathilake: None. **C. Brodbeck:** None. **S. Bhattasali:** None. **P. Resnik:** None. **J. Simon:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.02

Topic: D.05. Auditory & Vestibular Systems

Support: NIH/NIDCD R01DC018734

Title: Spontaneous EEG oscillations and cross-frequency coupling in very preterm infants

Authors: *C. BLANKENSHIP, M. BARNES-DAVIS, G. WESTERKAMP, R. LIU, K. CULLION, E. PEDAPATI, N. PARIKH, D. MOORE, L. HUNTER;
Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Very preterm infants (VPT; gestational age [GA] < 32 weeks) are at high risk for speech language impairments and permanent hearing loss. Continuous EEG can be used to measure spontaneous neural activity and functional connectivity between brain regions known to support speech and language development. Studies have shown that VPT infants have decreased oscillatory activity within the theta, alpha and beta bands, but equivalent delta activity, compared to full term infants. However, differences in spontaneous neural activity across brain regions and relationship with sub-cortical auditory function and hearing status in preterm infants have yet to be explored. This longitudinal study aims to improve early prediction of speech, language and pre-literacy deficits in VPT infants. It includes 150 infants who will be assessed from birth to 3 years with resting-state functional MRI, resting and speech-evoked EEG, hearing and speech-language measures. In this analysis, 46 VPT infants (24-32 weeks GA) were tested at 3 months corrected age (Range = 1-5 mos; male = 26). Spontaneous EEG was recorded while the infants were sleeping using a high-density net (128 channels; EGI, Inc). Relative power (delta, theta, alpha, beta, and gamma bands) and cross-frequency amplitude-amplitude coupling (CFC) between low frequency (theta, alpha) and high frequency (gamma) was calculated. Repeated measures ANOVAs examined differences in power and CFC across brain region topography and hemisphere. Length of NICU stay, GA, and age at test were included as covariates. Lateralization of maximal relative power varied by oscillatory band. Delta power had a right hemisphere preference, while theta-gamma power demonstrated a left hemisphere preference. Relative power varied by region with maximal delta power within the occipital/temporal areas and maximal alpha-gamma power within the central/frontal areas. CFC differed significantly between regions, with stronger coupling in the central/parietal regions and weaker coupling within the frontal/temporal/occipital regions. CFC was positively correlated with sub-cortical auditory function and length of NICU stay. These results are congruent with existing literature, but are highly novel as they are the first to characterize spontaneous EEG activity as a function of hemisphere and region and to explore relationships with sub-cortical auditory function in VPT infants. Ongoing analyses includes evaluation of EEG phase coupling and relationships between spontaneous EEG with MRI brain abnormality scores and physiological hearing thresholds.

Disclosures: C. Blankenship: None. **M. Barnes-Davis:** None. **G. Westerkamp:** None. **R. Liu:** None. **K. Cullion:** None. **E. Pedapati:** None. **N. Parikh:** None. **D. Moore:** None. **L. Hunter:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.03

Topic: D.05. Auditory & Vestibular Systems

Support: NRF-2022R1A2B5B02001752

Title: Emergence of music-selectivity in deep neural networks trained for natural sound detection

Authors: *G. KIM, D.-K. KIM, H. JEONG;
KAIST, KAIST, Daejeon-City, Korea, Republic of

Abstract: Music exists in almost every society, has universal acoustic features, and is processed by distinct neural circuits in humans even with no experience of musical training. These characteristics suggest an innateness of the sense of music in our brain, but it is unclear how this innateness emerges and what functions it has. Here, using an artificial deep neural network that models the auditory information processing of the brain, we show that units tuned to music can spontaneously emerge by learning natural sound detection, even without learning music. By simulating the responses of network units to 35,487 natural sounds in 527 categories, we found that various subclasses of music are strongly clustered in the embedding space, and that this clustering arises from the music-selective response of the network units. The music-selective units encoded the temporal structure of music in multiple timescales, following the population-level response characteristics observed in the brain. The music-selectivity of the network gradually increased throughout the training process for natural sound detection. Based on this, we confirmed that the process of generalization is critical for the emergence of music-selectivity and that music-selectivity can work as a functional basis for the generalization of natural sound. We found that ablation of the music-selective units significantly deteriorates the natural sound detection performance of the network. This suggests that music and other natural sounds share key features, and thus music-selective units can play a functionally important role not only in music processing but also in natural sound detection. Further investigation showed that training with speech helps the network units to acquire long-range temporal features of music. Our findings suggest that our sense of music can be innate, universally shaped by evolutionary adaptation to process natural sound.
https://www.biorxiv.org/content/10.1101/2021.10.27.466049v2

Disclosures: G. Kim: None. **D. Kim:** None. **H. Jeong:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.04

Topic: D.05. Auditory & Vestibular Systems

Title: Effects of Repetition of Naturalistic Music on Neural Encoding

Authors: *B. MANOLOVITZ[1], A. R. DYKSTRA[2], M. OGIHARA[3], C. GIBSON[1];
[1]Univ. of Miami, Miami, FL; [2]Dept. of Biomed. Engin., Univ. of Miami, South Miami, FL;
[3]Computer Sci., Univ. of Miami, Miami, FL

Abstract: How the brain encodes natural stimuli like speech and music is a fundamental question of auditory neuroscience. Here, we examined the neural encoding of music familiarity using electroencephalography and natural polyphonic music. 27 subjects (16 female, mean age = 20.43 ± 2.59) listened to 30-second song excerpts from mainstream music genres and provided liking and familiarity ratings. Each subject heard familiar and unfamiliar songs five times during the experiment. Neural responses were correlated to both the envelope and spectral flux of the stimuli using temporal response functions (TRFs), which use regularized ridge regression to learn optimal mappings between continuous stimulus features and neural responses. This predicted response is correlated to the true neural response to produce prediction accuracies, which are indicators of the extent to which the stimulus features are encoded in the neural responses. We hypothesized that the encoding of stimulus features would be stronger for familiar vs. unfamiliar music, and that the strength of the encoding of unfamiliar stimuli would increase with repetition. Pearson's r correlations between predictions and responses were analyzed based on two factors: familiarity with the excerpt and play count. Additionally, model weights were compared between familiarity conditions using support vector machines (SVMs). Overall, the prediction accuracies are lower than those from similar studies (Envelope: $r = 0.003$, Spectral Flux: $r = 0.0013$). There was no significant difference in the TRF prediction accuracies between familiar and unfamiliar trials for envelope ($t27 = 1.007$, $p = 0.16$), but there was for spectral flux ($t27 = 2.097$, $p < 0.05$). However, the SVM was able to distinguish between familiarity conditions using TRF model weights (Envelope: accuracy = 86.97%, Spectral Flux: accuracy = 87.91%). There was no significant effect of play count on prediction accuracy (all $F4,104 < 0.7$, all $p > 0.6$).

Disclosures: **B. Manolovitz:** A. Employment/Salary (full or part-time):; University of Miami. **A.R. Dykstra:** A. Employment/Salary (full or part-time):; University of Miami. **M. Ogihara:** A. Employment/Salary (full or part-time):; University of Miami. **C. Gibson:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.05

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01 DC012379
Howard Hughes Medical Institute

Title: Single neuron encoding of speech across cortical layers of the human superior temporal gyrus

Authors: *L. GWILLIAMS[1], M. K. LEONARD[1], K. K. SELLERS[1], J. E. CHUNG[1,2], B. DUTTA[2], E. F. CHANG[1];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]IMEC, Leuven, Belgium

Abstract: Decades of lesion and brain imaging studies have identified the superior temporal gyrus (STG) as a critical brain area for speech perception. Here, we used high-resolution multi-laminar Neuropixels arrays to record from hundreds of neurons in the human STG while participants listened to natural speech. We found that neurons exhibit tuning to complex spectro-temporal acoustic cues, which correspond to phonetic and prosodic speech features. However, single neuron activity across the cortical layers demonstrated a highly heterogeneous set of tuning profiles across the depth of the cortex, revealing a novel dimension of speech encoding in STG. Finally, single neuron speech-evoked responses across cortical layers were compared with field potentials recorded at the cortical surface. We found that high-frequency field potential activity reflects the contributions of neurons across all depths, encompassing the diversity of tuning response profiles across cortical layers. Together, these results demonstrate an important axis of speech encoding in STG, namely single neuron tuning across the cortical laminae.
*L.G. and M.K.L. contributed equally.

Disclosures: L. Gwilliams: None. **M.K. Leonard:** None. **K.K. Sellers:** None. **J.E. Chung:** None. **B. Dutta:** None. **E.F. Chang:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.06

Topic: D.05. Auditory & Vestibular Systems

Support: FWO SB Grant 1SF1322N

Title: Effects of language understanding on hemispheric lateralization in brain activity

Authors: *J. COOPER, M. GILLIS, L. VAN DEN EYNDE, J. VANTHORNHOUT, T. FRANCART;
Katholieke Univ. Leuven, Leuven, Belgium

Abstract: Motivation Previous research has shown that the left hemisphere of the brain plays an important role in language understanding. We investigate the effects of language understanding on hemispheric lateralization in brain activity using electroencephalography (EEG) and temporal

response functions (TRFs) in two experimental paradigms that use different languages. Using these languages we observe how activity in the brain changes depending on whether or not the listener understands the language.
MethodsIn a first study, 26 native Dutch-speaking subjects listened to a Dutch story and a Frisian story while their EEG was recorded. In a second study, 4 subjects listened to 8 stories (3 stories in Dutch, 3 in French, and 2 in Italian) while their EEG was recorded. For data analysis, we correlated the envelope of each stimulus with the corresponding EEG signal to generate a TRF for each channel in the EEG.
ResultsIn the TRFs, increased left-hemisphere lateralization was found at 150 ms when the listeners were presented with their first language (e.g. Dutch). When listeners were presented with their second language (e.g. French) there was decreased left-hemisphere lateralization at 150 ms. This decreased left-hemisphere lateralization was also observed when listeners were presented with languages they do not understand (e.g. Italian or Frisian).
ConclusionUsing EEG measurements, we analyzed the effects of language understanding on hemispheric lateralization in TRFs. The hemispheric lateralization observed when subjects listen to their second language is inconsistent with lateralization observed when listening to their native language. Therefore, hemispheric lateralization could be used to assess native language understanding for individuals with hearing impairment and/or neurological disorders (e.g. aphasia).

Disclosures: J. Cooper: None. **M. Gillis:** None. **L. Van den Eynde:** None. **J. Vanthornhout:** None. **T. Francart:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.07

Topic: D.05. Auditory & Vestibular Systems

Support: The Kavli Institute for Brain and Mind

Title: Probing the Black Box in Human Cognition and Machine Learning: Representation of Specialized Processing of Speech and Music in the Brain and Deep Neural Networks

Authors: *T.-H. Z. CHENG[1], K.-L. CHEN[2], J. SCHUBERT[5], T. T. BROWN[3], J. R. IVERSEN[4];
[1]Dept. of Cognitive Sci., [2]Dept. of Electrical and Computer Engin., [3]Dept. of Neurosciences, [4]Inst. for Neural Computation, UC San Diego, La Jolla, CA; [5]Univ. of Salzburg, Salzburg, Austria

Abstract: Human listeners can accurately recognize a vast number of complex sounds, but some, speech and music, are core to our identity as humans. While much is known about auditory coding of simple sounds, how complex sounds are represented is still unclear. It has

been suggested that speech and music may be selectively coded by the brain, differently from other complex sounds, but this remains controversial. In the past five years, deep neural networks (DNNs) have achieved human-level accuracy in sound classification, and may share some of the same representational properties as biological brains, starting a new and exciting trend of research using DNN as a parallel model to investigate neuroscientific principles. To date, few studies have compared DNNs to brain processing of dynamically changing complex auditory signals. In this study we compare the human brain and DNNs trained and tested using the same sound classification task, seeking to test if similar hierarchical principles exist in models and brains in order to further understand sound representation in the brain. Magnetoencephalographic (MEG) signals were recorded while subjects (N = 10) listened to speech, music and other complex sounds extracted from the Natural Sound Dataset. Neural networks with a variety of architectures with skip connections (e.g. ResNet-20, U-Net, WRN-40-4) and traditional feedforward convolutional neural networks were trained using the same dataset to classify complex sounds. Preliminary results showed a significantly higher decoding accuracy for speech ($t(9) = 2.87$, $p < 0.05$) and music ($t(9) = 2.74$, $p < 0.05$) than other sounds from MEG localized only to secondary auditory areas (Brodmann area 22) ($F(9,90) = 3.01$, $p < 0.005$). In contrast, there was no significant selectivity shown at the primary auditory cortex (Brodmann area 41/42) ($F(9,90) = 1.28$, $p = 0.26$). We further compared DNN model unit outputs to neural signals and found that the secondary auditory areas were generally predicted better by later DNN model layers compared to earlier layers. Our results suggest a higher cortical selectivity for speech and music in contrast to other complex sounds. These speech and music selective cortical regions could be explained better by the later, and more complex layers of DNN. This may suggest comparable hierarchical principles of DNN and neural processing of sounds. These preliminary results are compatible with special coding for speech and music in the brain, and this line of work could ultimately reveal architectural design for state-of-the-art neural networks used for processing complex sounds.

Disclosures: **T.Z. Cheng:** None. **K. Chen:** None. **J. Schubert:** None. **T.T. Brown:** None. **J.R. Iversen:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.08

Topic: D.05. Auditory & Vestibular Systems

Support: IIS-1912286
R01NS109367

Title: Distributed Feedforward and Feedback Cortical Processing Supports Human Speech

Authors: ***R. WANG**[1], X. CHEN[1], A. KHALILIAN-GOURTANI[1], P. DUGAN[2], L. YU[4], D. FRIEDMAN[5], W. K. DOYLE[6], O. DEVINSKY[2], Y. WANG[1], A. FLINKER[3];

[1]Electronic and Computer Engin., New York Univ., Brooklyn, NY; [3]Neurol., [2]New York Univ., New York, NY; [4]NYU Sch. of Med., New York City, NY; [5]New York Univ. Langone Med. Ctr., New York, NY; [6]NYU, New York, NY

Abstract: Speech production is a complex human function requiring continuous feedforward commands together with reafferent feedback processing. These processes are carried out by distinct frontal and temporal cortical networks, but the degree and timing of their recruitment and dynamics remain unknown. We present a novel deep learning architecture that translates neural signals recorded directly from cortex to an interpretable representational space that can reconstruct speech. We leverage novel learnt decoding networks to disentangle feedforward vs. feedback processing. Unlike prevailing models, we find a mixed cortical architecture in which frontal and temporal networks each process both feedforward and feedback information in tandem. We elucidate the timing of feedforward and feedback related processing by quantifying the derived receptive fields. Our approach provides evidence for a surprisingly mixed cortical architecture of speech circuitry together with decoding advances that have important implications for neural prosthetics.

Disclosures: R. Wang: None. **X. Chen:** None. **A. Khalilian-Gourtani:** None. **P. Dugan:** None. **L. Yu:** None. **D. Friedman:** None. **W.K. Doyle:** None. **O. Devinsky:** None. **Y. Wang:** None. **A. Flinker:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.09

Topic: D.05. Auditory & Vestibular Systems

Support: UNLV Top-Tier Doctoral Research Fellowship

Title: A Multi-Lab EEG Replication and Extension of “Tagging the Neural Entrainment to Beat and Meter”

Authors: *K. NAVE, E. E. HANNON, J. S. SNYDER;
Univ. of Nevada, Las Vegas, NV

Abstract: Cognitive neuroscienceresearch has attempted to disentangle stimulus-driven processing from consciousperceptual processing for decades. While some prior evidence for neuralprocessing of perceived musical beat (periodic pulse) may be confounded bystimulus-driven neural activity, a seminal finding in auditory neuroscience hasevidenced perception-related brain responses to imagined musical beat whileholding stimulus features constant. Frequency tagging, which measureselectrical brain activity at frequencies present in a stimulus, showedincreased brain activity at beat-related frequencies when listeners imagined ametrical pattern while listening to an isochronous auditory stimulus(Nozaradan, Peretz, Missal, & Mouraux, 2011). However, it is unclearwhether this represents repeatable evidence for conscious

perception of thebeat reflecting the population-level effect size, and whether this effect isrelated to relevant music experience, such as music and dance training. ThisRegistered Report (journal: Advances in Methods and Practices in PsychologicalScience) details the results of 13 independent conceptual replications ofNozaradan et al. (2011), all using the same pre-registered vetted protocol (seehttps://osf.io/d8fmb for more information onour pre-registration). Listeners performed the same imagery tasks as inNozaradan et al. (2011), with the addition of a behavioral task on each trialto measure conscious perception. Meta-analyses will examine the effect ofimagery condition and estimate the meta-analytic effect size for eachcondition, as well as the presence (or lack thereof) significant moderatingeffects of music and dance training. Logistic regression will estimate thepredictive value of behavioral performance on brain activity on individualtrials. With pre-registered data analysis culminating Summer 2022, results willbe presented at the conference. We will discuss possible explanations fordiscrepancies between these findings and the original study and implications ofthe extensions provided by this registered report.

Disclosures: **K. Nave:** 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.; UNLV Top-Tier Doctoral Research Fellowship. **E.E. Hannon:** None. **J.S. Snyder:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.10

Topic: D.05. Auditory & Vestibular Systems

Support: FWO research project G0D6720N
KU Leuven Special Research Fund C24/18/099
FWO postdoctoral fellowship 1290821N

Title: Relating linguistic speech features with EEG using a deep neural network

Authors: *C. PUFFAY[1,2], J. VANTHORNHOUT[1], M. GILLIS[1], B. ACCOU[1,2], H. VAN HAMME[2], T. FRANCART[1];
[1]Dept. Neurosciences, [2]Dept. Electrical Engin., KU Leuven, Leuven, Belgium

Abstract: The extent to which the brain tracks a speech stimulus can be measured for natural continuous speech by modeling the relationship between stimulus features and the corresponding EEG. Typically acoustic features are used, such as the temporal envelope, but recently neural tracking of lexical and linguistic features has also been shown. Subject-specific linear models have been used to predict EEG from acoustic, lexical, and linguistics features. As linguistics are processed in high-cortical areas, we expect their response to have a nonlinear component that cannot be modeled by a linear model.

Therefore, we present a deep learning model to obtain a nonlinear subject-independent model relating EEG to linguistic features.
This multi-input model evaluates the added value of linguistic features over and beyond acoustic and lexical information. Sixty normal-hearing subjects listened attentively to 10 stories of about 14 minutes each while their EEG was recorded. To evaluate the ability of our model to relate EEG to linguistics, we use a match-mismatch classification task. The model must choose whether a segment of brain signal matches the auditory stimulus that evoked it (matched) or a segment of the same length taken 1s after the matched end (mismatched).
We here define neural tracking as the ability of the model to associate EEG with speech, and we use the classification accuracy on the match-mismatch task to measure it.
We compare the baseline model (including speech envelope, onset envelope, phoneme onset, and word onsets) with a model including different linguistic representations in addition to the baseline (namely phoneme surprisal, cohort entropy, word surprisal, and word frequency). Using subject-specific fine-tuning on a subject-independent pre-trained model, we found significant linguistic tracking on top of acoustic and lexical tracking for some features.
We showed that some of the linguistic features carry additional information beyond acoustics and lexical features. The benefit of our deep learning model is that it may need less subject-specific training data than a linear model and that it can model non-linear relations between stimulus features and EEG. We will further use this model to objectively measure speech understanding.

Disclosures: **C. Puffay:** None. **J. Vanthornhout:** None. **M. Gillis:** None. **B. Accou:** None. **H. Van hamme:** None. **T. Francart:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.11

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD R01DC020097

Title: Modeling and predicting human perceptual sensitivity of speech recognition in natural environmental noise

Authors: *X. ZHAI[1], A. CLONAN[1], I. H. STEVENSON[2], M. A. ESCABI[1];
[1]Electrical and Computer Engin., [2]Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT

Abstract: Being able to recognize sounds in competing noise is a critical task of the normal functioning auditory system. Here we performed human psychoacoustic studies to assess how the spectrum and modulation content of natural background sounds masks the recognition of a speech digits (0-9). Native English speakers with normal hearing (0-20 dB threshold, 0.25-8 kHz) listened to digits in various original and perturbed maskers (e.g., water, bird babble,

construction noise, speaker babble, etc.) at 72 dB SPL and -9 dB SNR. Phase randomized (PR, retains the spectrum but distort the modulations) and spectrum equalized (SE, preserves the modulations and distort the spectrum) backgrounds variants were also used to dissociate spectrum vs. modulation masking effects. Response accuracy shows differences across sounds and across conditions indicating that some of the masking can be attributed to the modulation content and its high-order structure. For instance, the PR speech babble exhibits an increase in the accuracy, indicating that the modulation content is a major masking component. For construction noise, by comparison, the modulations tend to improve the accuracy. Thus, individual backgrounds can produce varied outcomes and the unique modulation content of each background can affect digit identification beneficially or detrimentally. We next developed an auditory midbrain model to determine whether masker interference in a physiologically inspired modulation space could predict the perceptual trends. Sounds were decomposed through a cochlear filterbank and a subsequent set of spectro-temporal receptive fields that model modulation sensitivity and map the waveform into temporal and spectral modulation. These outputs were then sent to a logistic regression model which we use to estimate perceptual transfer functions and to ultimately predict response accuracy. Cross validated predictions demonstrate that the model accounts for ~90% of the perceptual response variance. The model also outperformed predictions obtained using a cochlear model, which accounted only for ~ 60 % of the variance. The perceptually derived transfer functions subsequently allow us to identify salient cues that impact recognition in noise. For instance, slow background modulations (<8 Hz) tended to reduce accuracy whereas spectral modulations in speech in the voicing harmonicity range tended to improve accuracy. The finding demonstrate that the modulation content of environmental sounds can have adversarial masking outcomes on speech recognition and that an auditory midbrain inspired representation can predict and identify high-order cues that contribute to listening in noise.

Disclosures: **X. Zhai:** None. **A. Clonan:** None. **I.H. Stevenson:** None. **M.A. Escabi:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.12

Topic: D.05. Auditory & Vestibular Systems

Support: Samsung Research Funding & Incubation Center of Samsung Electronics under Project Number SRFC-IT1902-08

Title: Neural correlates of auditory features during music imagery

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Abstract: Music requires the human brain to process a variety of musical properties, such as rhythm-related note onset timing, and melody consisting of pitch series. Previous neuroimaging studies have identified various brain areas involved in music imagery. Recent studies showed that it is possible to confirm note onset timing during musical imagination using cortical activities. However, it is still unknown how pitch-related information is represented in the brain. Here, we decoded the seven distinct notes (Do-Re-Mi-Fa-Sol-La-Ti) of a diatonic scale using brain activities considering that the general population is the relative pitch. We recorded electrocorticography signals with three intractable epilepsy patients and subjects were instructed to imagine the familiar children's song. In order to obtain precise time-locking neural activity with the note onset timing, by presenting the listening conditions which listening to the first part of the song before every imaging condition, we induced the subject to imagine the song at the same beat as the listening conditions (120 bpm; inter-onset timing 500 ms). We classified 7 components (Do-Re-Mi-Fa-Sol-La-Ti) of a diatonic scale as gamma activity (30-150 Hz) during imagining a single note using an error-correcting output code (ECOC) multiclass model based on support vector machines.We found a significantly increased gamma response during imagery conditions in superior temporal gyrus, middle temporal gyrus, and sensorimotor areas ($p<0.05$ Upper tailed t-test). Using the gamma power (30-150 Hz) in these areas, we could obtain mean decoding accuracy (40.1%) upper than two times of chance level(14.3%). In addition, the accuracy was different depending on the song, which seemed to be less accurate in familiar songs.In this study, we showed that the decoding of the pitch domain using ECoG signals in areas activated during music imagery is possible. This result provide based on the developing music BCI system.

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Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.13

Topic: D.05. Auditory & Vestibular Systems

Title: Neural encoding of melody to pitch less sounds

Authors: *A. SANTOYO, M. G. GONZALES, Z. J. IQBAL, K. C. BACKER, R. BALASUBRAMANIAM, H. BORTFELD, A. J. SHAHIN;
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Abstract: Previous research has demonstrated that melodic processing activates regions of the superior temporal gyrus (STG) and planum temporale (PT) in the temporal lobe. Research done by Patterson and colleagues used functional magnetic resonance imaging to map the perceptual processing of white noise as it was perceived with no pitch, pitch, and melody, without changing the acoustic properties of the white noise. They found that as perception shifted from experiencing no pitch to a melody, patterns of activation shifted anterolaterally from Heschl’s

gyrus (HG) to the right STG and PT (Patterson et al., 2002). Moreover, several studies have similarly demonstrated that there is a hierarchy in regions of activation as sounds become more complex. Previous work using electroencephalography (EEG) study found that melodic perception triggers stronger phase-locking of neural activity to sounds than in speech perception (Liberto et al., 2020). Here, we use EEG to extend and advance our neurophysiological knowledge of melody processing without the influence of pitch by using sounds with no perceived pitch. We constructed these stimuli with seven 200 ms segments of white, brown, and pink noise separated by 50 ms of silence, to form nonmelodic (NM) and melodic (M) streams. In NM streams, all seven noise segments were of the same type of noise. The M streams, on the other hand, comprised a patterned sequence with all three types of noise present in each stream. If successful, the M streams should give the perception of a melody in listeners due to spectral variation across noise segments and induce a music-like experience. Participants were asked to rate M and NM streams as sounding musical or non-musical for ten blocks during neural recordings. Preliminary data (n = 7) showed that melody encoding is manifested in stronger phase-locking to noise bursts at frontocentral sites, indicative of an auditory neural source being more spectrally tuned to the stimuli during M versus NM perception. Time-frequency decomposition also showed increased gamma power at lateral frontocentral sites, indicative of engagement of higher-level auditory networks during melody encoding.

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Poster

633. Auditory: Human Speech and Music

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH-NIDCD 5R01DC014279

Title: Cortical Encoding of Long-Term Temporal Dependencies in Music Perception

Authors: *Y. LI[1], P. M. PATEL[1], S. BICKEL[2], A. D. MEHTA[3], N. MESGARANI[1];
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Abstract: The human auditory system is responsive to temporal dependencies that are an integral part of time-series signals such as music. How the musical context at different timescales is encoded in various regions of the human auditory cortex and how musical expertise affects the encoding of context remain unclear. To shed light on these questions, we used a transformer neural network known for its contextualization over layers to model musical pieces at different timescales. The 12-layer sequence-to-sequence multi-tasking transformer model was trained on music MIDI data with both an encoder and a decoder. The encoder was trained to predict the

masked input values, and the decoder was trained to predict the next note given all previous notes. Transformer features were derived from different layers of the encoder and were used to predict neural responses recorded either with scalp or intracranial EEG. Scalp EEG was recorded from 10 expert pianists (musicians) and 10 subjects without musical training (non-musicians). The iEEG data was recorded from 5 neurosurgical patients undergoing epilepsy surgery. All subjects listened to 30 minutes of music consisting of 8 Bach pieces. We applied a non-negative matrix factorization (NMF) transformation on features from each layer of the encoder of the transformer model to reduce the dimensionality and use them to linearly predict the EEG and iEEG responses using leave-one-out cross-validation. We found that the prediction correlations were monotonically increasing over the layers of the transformer encoder for both EEG and iEEG data ($p < 0.05$). The differences in correlation also increased more for musicians compared to non-musicians. We further performed k-means clustering of iEEG electrodes based on their correlation over layers and identified two major clusters with different correlation/layer progressions across electrodes. The first cluster of electrodes showed increased correlation which plateaued after layer 5. The electrodes in this cluster were mostly located in the transverse temporal sulcus (TTS), Heschl's gyrus (HG), and planum temporale (PT). The second cluster of electrodes showed increased correlation which did not plateau before layer 8, with electrodes in the lateral aspect of the superior temporal gyrus (STG), middle temporal gyrus (MTG), and inferior frontal gyrus (IFG). These findings show the efficacy of deep learning models in revealing the multiscale pattern of music encoding in the human auditory system, demonstrate differences in how musical context is processed in musicians and non-musicians, and suggest a hierarchy in contextual processing of music across various brain regions.

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Poster

633. Auditory: Human Speech and Music

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Program #/Poster #: 633.15

Topic: D.05. Auditory & Vestibular Systems

Support: JSPS Kakenhi 21K18311

Title: Alpha power in the prediction of familiar melodies

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Abstract: Introduction: Melody and language share similar characteristics in their processing [1]. Typical language processing is predictability; it has been reported that event-related fields occur during word prediction, and alpha power (8-12 Hz) strongly suppresses when words are easy to predict [2]. This study hypothesized a similar mechanism in predicting melody as in

language; alpha power is more suppressed when the melodies are more predictable than when they are less predictable. Methods: Twenty healthy young adults (ten males, mean age 22.1 ± 1.51; range 20-27 years old) participated in the experiment. All participants self-reported normal hearing. They gave their written informed consent, and the research ethics committee approved the study in Tokyo University of Agriculture and Technology. In this experiment, we measured the EEGs while listening to a melody with a silent section while imaging. At the end of each melody, the participants were asked whether they were familiar with the presented melody. In data analysis, first, we divided the filtered EEG (1-30 Hz) into two groups: familiar and unfamiliar melodies. Next, we compared the two groups' event-related potentials (ERPs) to explore the brain response during the silent section. Moreover, we performed a time-frequency analysis and compared the alpha power fluctuations between the familiar and unfamiliar groups. Results: We found no significant difference in ERPs between the two groups of familiarity. Furthermore, we observed stronger alpha power suppression before the silent section for familiar melodies compared with unfamiliar ones in the left frontal and central regions. Discussion: The results suggest that ERPs were elicited when trying to predict melodies, and alpha power suppression reflects the predictability of melodies. In addition, we suggest that melody and language follow similar processes, further strengthening the similarities between the two domains. References: [1] Miranda et al., NeuroImage, 2007 [2] Wang et al., Journal of Cognitive Neuroscience, 2018

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Poster

633. Auditory: Human Speech and Music

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Topic: D.05. Auditory & Vestibular Systems

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Title: Processing of auditory semantic novelty in human cortex: An intracranial electrophysiology study

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Abstract: Semantic novelty paradigms have been useful tools to probe the cortical circuits involved in language processing. Neuropsychiatric disorders, including autism, schizophrenia, and disorders of consciousness, are characterized by aberrant detection of semantic novelty. The current investigation took advantage of the superior spatio-temporal resolution of intracranial

electroencephalography (iEEG) to examine semantic novelty processing a large cohort of subjects with comprehensive electrode coverage. Subjects were adult neurosurgical patients (N = 38; 18 women) undergoing chronic invasive monitoring for medically intractable epilepsy. Cortical activity was recorded using depth and subdural electrodes (over 5700 contacts total), with extensive coverage of lateral temporal, frontal, parietal and limbic cortex. Stimuli were monosyllabic words from three semantic categories, presented in auditory target detection tasks. Each task included common words “cat”, “dog”, “five”, “ten”, “red”, and “white” (20 exemplars each), and ten novel words, five of which were in the target category. Cortical activity was measured as event-related band power in broadband gamma (30-150 Hz) and alpha (8-14 Hz) bands (reflecting feedforward activation and release from feedback inhibition, respectively), and averaged evoked potentials (AEPs). Effects of semantic novelty were measured as differences between responses to common and novel words. Linear mixed effects models were used to assess regional and hemispheric differences in novelty effects while accounting for across-subject heterogeneity. Semantic novelty was associated with greater task difficulty, as indexed by lower target hit rates and longer reaction times. Responses to novel words (augmented gamma power and more pronounced suppression of alpha power) were broadly present in the cortex, particularly in rostral temporal, limbic, and prefrontal areas. The most prominent AEP effects were observed in the left anterior temporal lobe. There was a complex pattern of hemispheric asymmetries, with either left- or right-hemisphere bias evident for different brain areas and iEEG frequency bands. This study provides a framework for understanding activation patterns of brain areas involved in processing of auditory semantic novelty. Pronounced novelty effects occur in anterior and medial temporal areas, which serve as global network hubs (Banks et al., bioRxiv 2022.02.06.479292) involved in higher-order sensory and speech processing. The current study helps lay the foundation for further clarifying aberrant speech and language processing in clinical neuropsychiatric populations.

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Poster

633. Auditory: Human Speech and Music

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Topic: D.05. Auditory & Vestibular Systems

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CArver Charitable TRust RPOE

Title: Long-term neurophysiological impact and compensation after disconnection of the anterior temporal lobe

Authors: *Z. KOCSIS[1], J. I. BERGER[1], R. M. CALMUS[2], B. MCMURRAY[1], M. E. SARRETT[3], P. E. GANDER[1], C. K. KOVACH[1], J. D. GREENLEE[1], A. D. BOES[1], T. NICKL-JOCKSCHAT[1], H. KAWASAKI[1], I. CHOI[1], M. A. HOWARD, III[1], C. I. PETKOV[2];
[1]Univ. of Iowa, Iowa City, IA; [2]Newcastle Univ., Newcastle upon Tyne, United Kingdom; [3]Villanova Univ., Villanova, PA

Abstract: A fundamental question in neuroscience is the human brain's capacity for plasticity and how it reorganizes and compensates for impact on its neural systems. Recently, rare intracranial ECoG recordings were reported, conducted tens of minutes before and after a neurosurgical resection procedure that required disconnection of the left anterior temporal lobe (ATL) to treat intractable epilepsy [1]. The results, obtained during a speech semantic expectation task, showed immediate neural system impact on speech sound processes and prediction, providing direct causal evidence for the importance of the ATL as a semantic hub [2]. The results also showed remarkable compensation in the form of increased functional interconnectivity between language-critical areas in inferior frontal cortex and speech processing sites in auditory cortex. Here, we report source-localized high-density EEG results with the same task in the two left hemisphere ATL disconnection patients previously reported and an additional right hemisphere ATL disconnection patient. The EEG data were obtained 2-6 weeks before and 2 and 6-14 months after their surgical treatment procedure. Our aims here were to study, 1) the correspondence between the ECoG and EEG in the same patients, and; 2) longer-term compensation throughout the brain, which EEG coverage extends beyond the treated hemisphere. Behavioral data on the task assessed pre- and post-operatively showed a striking post-surgical impact on speech perception and prediction in the left hemisphere ATL patients, but a lack of such an effect in the right hemisphere ATL patient whose speech perception and prediction ability stayed within the normative range of control participants. In all three patients, the source-localized EEG signals after ATL disconnection showed magnified frontal (inferior frontal gyrus, pars opercularis) and auditory cortical (Heschl's gyrus and superior temporal gyrus) responses to the speech sounds in the hemisphere affected by the surgical procedure, recapitulating key effects observed immediately after the surgical procedure in the intracranial ECoG recordings [1]. Moreover, the contralateral hemisphere showed magnified responses to the speech sounds post-disconnection only when the left hemisphere was affected. The overall results both establish key correspondences between the ECoG and source-localized EEG signals and identify forms of compensation evident with EEG that can now be assessed in many patients not limited to these types of rare procedures. [1] Kocsis et al. (2022) https://doi.org/10.1101/2022.04.15.488388
[2] Rogers & Lambon Ralph (2022) https://pubmed.ncbi.nlm.nih.gov/35090837

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Poster

633. Auditory: Human Speech and Music

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Program #/Poster #: 633.18

Topic: D.05. Auditory & Vestibular Systems

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Title: Understanding rat behavior in a complex task via non-deterministic policies

Authors: *J. NIEDIEK[1], M. M. JANKOWSKI[1], A. POLTEROVICH[1], A. KAZAKOV[1], I. NELKEN[1,2];
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Abstract: Animal experiments in neuroscience often use simple, coarse measures of behavior. For example, trial outcome (correct/incorrect) is sometimes the only behavioral variable considered. However, this does not capture the complexity of animal behavior, with trials consisting of many animal actions. We developed a framework to study the behavior and simultaneous neuronal activity of freely moving rats at high resolution. We model behavioral tasks as Markov Decision Processes (MDPs), where a rat trajectory is described as a sequence of environment states and rat actions, with state transitions depending on current state and action. We computed deterministic optimal policies (a policy is a rule that prescribes an action in each state). In order to describe non-deterministic rat behavior, we computed non-deterministic, information-limited policies that realize optimal reward rates at a prescribed amount of deviation from non-informative behavior, quantified as the Kullback-Leibler divergence from a default, non-informative policy (Tishby's complexity, TC).
We applied our framework to data from five female rats performing a complex auditory-guided task, implemented in a large environment (diameter 160 cm) equipped with twelve nose-poke ports and loudspeakers to convey information to the rats. Rats had to position themselves at specific locations indicated by sounds to obtain reward. Despite the nontrivial task, rats reached high success rates within two 70-minute sessions. Observed rat trajectories resembled optimal policies derived from the model, but were non-deterministic. We estimated the TC of rat movement and nose-poking and its change over time by comparing rat behavior with information-limited policies.
Our model revealed a prolonged, large increase in the TC over time. Significantly, this prolonged behavioral refinement was not discernible via reward rates, and to our knowledge has not been described previously. The model also captured individual propensities for preferring some foraging strategies over others, as well as a reduction in the tendency of rats to perform nose-pokes that do not result in reward.
Recording with chronically implanted silicon probes from the left insular cortex, we found that in many neurons, firing rates (averaged over ten minutes) strongly correlated with TC in the same time periods. The proportion of highly correlated units was significantly larger in real recordings than in surrogate data.
Our model is based on first principles of information theory rather than on ad-hoc measures of

behavior. Measures derived from this model bring new insights into rat behavior, and seem to be reflected in rat brain activity.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

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Title: Coordinated neuronal ensembles enhance signal-in-noise processing in the auditory cortex

Authors: *N. Y. HOMMA[1], C. E. SCHREINER[2];
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Abstract: In the auditory cortex, groups of neurons show reliable synchronous activities (coordinated neuronal ensembles; cNEs). How individual neurons fire together to encode sensory information is, however, not well understood. Here we examined encoding of vocalizations in the presence of background noise in two core rat auditory cortical fields, primary auditory cortex and ventral auditory field (Sprague-Dawley rats, female, ~2-5 months). We obtained dense extracellular recordings using multichannel silicone probe and presented short segments of vocalizations embedded in various types of spectrotemporally modulated noise at six different signal-to-noise ratios. cNEs were identified based on dimensional reduction techniques (Lopes-dos-Santos et al, 2013; See et al., 2018), and the ability of decoding vocalizations or noise types was estimated by nearest-neighbor linear decoder (Foffani and Moxon, 2004) for cNEs and individual cortical neurons, respectively. The results suggest that (i) cNEs are more likely to decode vocalization and/or noise than single units, (ii) decoding accuracy of cNEs is improved compared to that of single units, and (iii) improvement of cNE decoding is most prominent for low level noise. These findings indicate that synchronous activities in the cortex could help refine spiking patterns and reduce the effects of noise in vocalization-in-noise processing. In addition, cNEs may contribute to emerging stimulus selectivity towards downstream areas, which support the idea that cNEs are a functional unit to enhance and convey essential sensory information.

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Poster

633. Auditory: Human Speech and Music

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Title: Automatic, unsupervised training of non-human primates on visuo-acoustic tasks

Authors: J. CABRERA MORENO[1,4,6,2], L. JEANSON[1,3], J. G. GRUNENBERG[1], T. MOSER[2,6,4,7,5], A. CALAPAI[1,8,3], ***M. JESCHKE**[1,6,8,2];
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Abstract: Automatic training of non-human primates (NHP) with touchscreen based systems directly in their home cage and social group has important applications in: phenotyping models of psychiatric and neurodegenerative diseases, in training and assessing cognition, as well as for the enhancement of animal welfare. In earlier work, we trained Rhesus macaques (*Macaca mulatta*) and Common marmosets (*Callithrix jacchus*) with step-wise unsupervised protocols that monitor the animal's performance and adjust the task difficulty accordingly. We showed that marmosets can discriminate conspecific vocalizations from artificial stimuli in a 2 or 3 alternative choice task directly in their home-cage without the need for food or water restriction nor social separation.In the current experiments, we expanded on this work by: 1) training the detection of artificial auditory stimuli in marmosets; and 2) training of a 3rd non-human primate species commonly used in pharmaceutical research (long-tailed macaques [*Macaca fascicularis*]) with the approach established with marmosets. First, marmosets were trained to expect the delivery of reward after the presentation of a pure tone. This allowed to devise a Go-NoGo task were animals had to trigger a pure tone by touching a screen once, or touching it twice when the initial touch did not result in a sound being played. Based on this approach, we gathered audiograms similar to published setup-based approaches. Second, two groups of socially housed long-tailed macaques were trained in daily sessions. Animals were identified with computer-vision based machine learning techniques allowing for individualized training. We found that animals 1) consistently engaged with the device across several months; 2) interacted in bouts of high engagement; 3) alternated peacefully to interact with the device; and 4) smoothly ascended from step to step in the visually-guided section of the procedure, in line with previous results.

However, we also found 4) that animals' performance remained at chance level as soon as the acoustically-guided steps were reached; and 5) that the engagement level decreased significantly with decreasing performance during the transition from visual to acoustic.Taken together, we conclude that it is possible to train non-human primate species directly in their social group and without dietary restriction, to solve a visually-guided discrimination task but not necessarily an acoustically-guided task with autonomous approaches. Our work lays the ground for future studies into cognition in NHPs and might be used in the assessment of cognitive effects in drug discovery research.

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Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.21

Topic: D.05. Auditory & Vestibular Systems

Title: Modulating the octave illusion in musicians and non-musicians

Authors: *P. GERMAIN[1,2], T. AUGEREAU[1,2], B.-A. BACON[3], F. CHAMPOUX[1,2], A. SHARP[4];
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Abstract: The octave illusion is a well-known auditory effect elicited by presenting a dichotic sequence of two tones separated by an octave, with the high and low tones alternating between both ears. To date, very few studies have investigated the octave illusion among professional musicians. It has been reported that even among the most highly trained musicians, only a small minority perceived the true physical stimulus instead of an illusory percept. Previous studies, however, have only used the central frequencies of the musical spectrum to elicit the illusion. A recent study from our laboratory suggests that the use of lower or higher frequency pairs can modulate the octave illusion in normal individuals. Whether such modulation is identical in musicians remains undetermined. The present study aimed at investigating how the relative frequency distribution of percepts changes across a larger range of the musical scale in a group of professional musicians in comparison to non-musicians. Twenty non-musician adults and 20 professional musicians were presented with 7 pairs of octave-separated frequencies ranging from 40-80Hz to 2000-4000Hz and had to select the choice that best corresponded to their perception: 1) A high-pitched sound on the right alternating with a low-pitched sound on the left; 2) A high-pitched sound on the left alternating with a low-pitched sound on the right; 3) A sound that passes from one ear to the other without a change in pitch; 4) none of these answers. Responses were divided in three categories: octave (answers 1 and 2), simple (answer 3) and complex

(answer 4). Results suggest differences in the response pattern as a function of the frequency pairs used to elicit this illusion between non-musicians and musicians. The data suggest an impact of musical training on the illusory percept and may help shed some light on the perceptual and neurophysiological correlates of musical training when presented with ambiguous stimuli.

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Poster

633. Auditory: Human Speech and Music

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Program #/Poster #: 633.22

Topic: D.05. Auditory & Vestibular Systems

Title: The role of self-timbre in pitch perception and imitation

Authors: *Y. CHEN, P. Q. PFORDRESHER;
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Abstract: Vocal imitation of pitch (e.g., during singing) depends on accurate and precise associations between auditory perception and vocal motor control. These associations lead to an advantage that has been found when humans imitate recordings of themselves as opposed to recordings of others (Pfordresher & Mantell, 2014). Given the close connection from perception to action, it is possible that this self-advantage stems from higher perceptual sensitivity of one's own voice. However, prior work failed to find such advantages in typical pitch discrimination tasks that compared pitches with the same timbre, and this discrepancy was considered a dissociation between perception and production (Moore et al., 2008). In this study, we provide an alternative account for the lack of self-advantage in pitch perception. We propose that the association between pitch perception and production is only measurable using a different perceptual discrimination task. It is known that auditory feedback plays a vital role in vocal production, and individuals use their own produced sound in real-time as feedback. Therefore, the relevant perception task in the imitation process is to compare sounds with different timbres; specifically sounds associated with the timbre of one's own voice as opposed to sounds associated with other sources (e.g., pure tones). We hypothesize that the efficiency of this cross-timbre comparison impacts vocal imitation performance. To test this hypothesis, we presented sounds with different timbres to participants and asked them to perform a pitch discrimination task. At the beginning of each session, participants were asked to sing a pitch within their comfortable vocal range. This comfort pitch was used to generate a series of self-timbre tones. Participants then completed perceptual pitch discrimination tasks involving different stimulus pairs: self-timbre vs. self-timbre, synthesized vs. synthesized, and self-timbre vs. synthesized. The first two conditions are considered timbre-matched, whereas the third one is timbre-mismatched. Preliminary results showed lower sensitivity and higher perceptual bias in the

timbre-mismatched condition (self-timbre vs. synthesized) compared with the timbre-matched conditions (self-timbre vs. self-timbre and synthesized vs. synthesized). This implies that comparing auditory feedback with the target during pitch imitation is more difficult than comparing sounds with the same timbre in typical pitch discrimination. Our study explains the relationship between pitch perception and production regarding self-advantage, and we demonstrate an interaction between pitch and timbre in pitch processing.

Disclosures: Y. Chen: None. **P.Q. Pfordresher:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 633.23

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01DC018055

Title: Predictive Coding in Auditory Cortical Neurons of Songbirds

Authors: *S. RUDRARAJU[1], B. H. THEILMAN[2], M. TURVEY[1], T. GENTNER[3];
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Abstract: Predictive coding (PC), a theoretical framework in which the brain compares a generative model to incoming sensory signals, has been employed to explain perceptual and cognitive phenomena, and has inspired computational models. There is little understanding, however, of how PC might be implemented at a mechanistic level in individual neurons within the auditory system. Here, we examined neural responses in caudomedial nidopallium (NCM) and caudal mesopallium (CM), analogs of higher order auditory cortex, in anesthetized European starlings listening to conspecific songs. We trained a simple, feedforward, temporal prediction model (TPM) (Singer et al., 2018) to predict short segments (10.5 ms) of future natural birdsongs based on past 170 ms spectrographic samples to generate a "latent" predictive feature space comprising 256 hidden units. The corresponding feature space computed by the mean squared error between the output of TPM (i.e. predicted song) and the true song represented prediction error. To examine PC, we modeled each neuron's composite receptive field (CRF), using the Maximum Noise Entropy method (MNE; Kozlov et al., 2016, Fitzgerald et al., 2011), in which the stimulus representation was either the short-time Fourier transform spectrogram of conspecific song, the projection of the spectrogram into a TPM latent space, or the mean squared error between TPM-predicted future spectrogram and the true spectrogram. This yields a version of neuron's CRF fit to either: 1) all spectrotemporal features (fft-CRF) or 2) only the predictive spectrotemporal features (tpm-CRF) or 3) prediction error spectrotemporal features (mse-CRF). In NCM (n = 541 neurons), the tpm-CRFs yield very good predictions of each neuron's empirical spiking response to novel song, accounting for 70.41% of the variance which is

slightly higher than the fft-CRF (67.92%; p< 5.5x10-8, paired t-test). The mse-CRFs yield significantly poorer predictions of responses to novel songs, explaining only 11.15% of variance (p=0.0, paired t-test). In CM (n = 137 neurons), as in NCM, the tpm-CRFs provide very good predictions of spiking responses similar to those of the fft-CRFs (77.36% and 79.20% variance explained, respectively; p< 1.0x10-15 paired t-test). Unlike NCM, however, the mse-CRF predicted a significant proportion of the CM response variance, 53.61% (p< 1.7x10-190, t-test CM vs NCM). We show that NCM spiking responses are best modeled by the predictive features of spectrotemporal song, while CM responses capture both predictive and error features. This provides strong support for the notion of a feature-based predictive auditory code implemented in single neurons in songbirds.

Disclosures: S. Rudraraju: None. **B.H. Theilman:** None. **M. Turvey:** None. **T. Gentner:** None.

Poster

633. Auditory: Human Speech and Music

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Program #/Poster #: 633.24

Topic: D.05. Auditory & Vestibular Systems

Support: Internal funds

Title: Dynamic encoding of phonetic categories in zebra finch auditory forebrain

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Abstract: To process information in a dynamically changing environment, our sensory systems need to adaptively encode salient sensory signals while de-emphasizing variations in those signals so as to form invariant representations. This capacity for perceptual invariance is crucial for vocal communication; humans form acoustic categories for human speech phonemes across speakers and exemplars. Other animals categorize conspecific vocalizations and can learn to categorize speech phonemes. The invariant representations of acoustic signals appear to emerge hierarchically in the auditory system. The present study investigated the neural representations of phonetic categories from human speech in zebra finch auditory forebrain and the influence of passive familiarization on those representations. Extracellular neural responses in the secondary auditory area NCM were recorded while zebra finches were passively exposed to two naturally spoken Dutch words produced by different human speakers. One group of birds was first exposed to the two word stimuli, and then tested with the same two words produced by novel human speakers. The other group was presented with the testing stimuli directly. Neural responses in NCM showed adaptation to repeated stimuli, indicating an effect of familiarity. Furthermore, population responses in NCM showed improved representation for word categories after passive familiarization. Decoding-based approaches showed that NCM neurons can form a

generalized representation for word categories independent of individual variations, and that the discrimination between word categories increases with passive familiarization in a subset of neurons. This study provides insights into the neural mechanisms of categorical representation and the dynamic encoding process underlying the formation of generalized representations.

Disclosures: W. Liu: None. **D.S. Vicario:** None.

Poster

633. Auditory: Human Speech and Music

Location: SDCC Halls B-H

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Program #/Poster #: 633.25

Topic: D.05. Auditory & Vestibular Systems

Title: Neural correlates of auditory-motor entrainment and synchronization in healthy adults: A scoping review of neuroimaging research

Authors: *M. PRANJIć[1,2], M. TAN[1], J. E. TEICH[1], L. TREMBLAY[1], M. H. THAUT[1]; [1]Univ. of Toronto, Toronto, ON, Canada; [2]Holland Bloorview Kids Rehabil. Hosp., Toronto, ON, Canada

Abstract: Auditory-motor entrainment occurs when the frequency of external rhythmic auditory cues determines the frequency of brainwave activity in the motor system. On a behavioral level, the addition of rhythmic auditory cues has been shown to enhance motor performance in patients with movement disorders. Recent findings show new evidence for an emerging understanding of neural mechanisms underlying rhythmic entrainment. Given its increased use in clinical applications, we aimed to systematically review neuroimaging research pertaining to auditory-motor coupling and its neural correlates. The scoping review was carried out across four major databases (Medline, Embase, PsycInfo, and Scopus) in accordance with the PRISMA-ScR guidelines. Studies were screened by two independent reviewers and had to (a) employ brain imaging and/or stimulation methods, (b) employ the finger-tapping paradigm, (c) involve healthy adults, and (d) be published in English before June 2022. From an initial return of 1430 records, 25 studies met the inclusion criteria and were included in the analysis. The studies employed six brain imaging and stimulation methods to unravel key neural mechanisms underlying auditory-motor entrainment. First, auditory-motor coupling relies on widely distributed cortical and subcortical neural networks, including the cerebellum, inferior colliculus, basal ganglia (BG), supplementary motor area (SMA), premotor cortex (PMC), and inferior frontal gyrus (IFG). Specifically, activations in the SMA, PMC, and BG were greater during self-paced tapping. Self-paced tapping also led to additional activations in the dorsolateral prefrontal cortex and IFG, suggesting that tapping in the absence of auditory cues requires more cognitive effort. Further, there seems to be a task-dependent non-linear relationship between the BOLD signal and beta oscillations within the SMA, where BOLD activity increases and power in the beta band decreases during auditory-cued finger-tapping. Finally, experimentally induced transient perturbations of the right dorsal PMC affected the synchronization accuracy, suggesting that

dPMC is crucial for rhythmic auditory-motor synchronization. Collectively, these studies provide insight into the neural substrates supporting the transformation of auditory input into timed rhythmic motor output. This is of particular interest to researchers due to its therapeutic applications in movement rehabilitation. Therefore, auditory-cued motor performance elicits significantly different neural pathways than self-paced movement, thus offering a possible means to circumvent various neural insults.

Disclosures: **M. Pranjić:** None. **M. Tan:** None. **J.E. Teich:** None. **L. Tremblay:** None. **M.H. Thaut:** None.

Poster

633. Auditory: Human Speech and Music

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Program #/Poster #: 633.26

Topic: D.05. Auditory & Vestibular Systems

Support: University of Connecticut Brain Computer Interface Core

Title: Sensory Discrimination and Cognitive Abilities: A Systematic Review and Meta-Analysis

Authors: *M. B. JANÉ[1], T. J. HARLOW[1], B. T. JOHNSON[1], H. L. READ[1,2];
[1]Psychological Sci., [2]Biomed. Engin., Univ. of Connecticut, Storrs, CT

Abstract: In the year 1904, Charles Spearman published the first empirical account of the relationship between higher-order cognitive and lower-order sensory discrimination ability. Since then, 118 years of research on the subject has been conducted without a meta-analysis to confirm the consistency and nature of this relationship. In this systematic review and meta-analysis, we investigate how performance on pitch discrimination tasks covary with higher-order cognitive tasks such as: working memory (digit span), verbal comprehension (vocabulary), and visuo-spatial/inductive reasoning (matrix reasoning). Additionally, we re-analyze correlation matrices that contain both sensory discrimination (across multiple modalities) and cognitive variables. These correlation matrices are fit to structural equation models, capturing the underlying cognitive and sensory abilities that accounts for the covariation in task performance. Lastly, we conduct in-depth re-analyses of large sample studies to confirm the robustness of our results. In particular, we examine how sensory modality, working memory and attentional demands account for these performance covariations. In sum, our meta-analysis indicates that sensory discrimination ability strongly covaries with general and specific cognitive abilities in humans. Together with what is known about cortical networks engaged, these results suggest that memory and attentional demands in sensory discrimination tasks can differentially engage cortical feedback pathways from higher level frontal, temporal or parietal cortices.

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Poster

634. Visual Processing During Behavior

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Program #/Poster #: 634.01

Topic: D.06. Vision

Support: Fondecyt 1210169
Fondecyt 3220871

Title: Development of the binocular visual field in a diurnal precocial rodent, the Octodon degus

Authors: *A. DEICHLER[1], M. RUIZ-FLORES[2], L. LOPEZ-JURY[2], C. GUTIÉRREZ-IBÁÑEZ[5], N. I. MÁRQUEZ[3], C. MORALES[2], J. MPODOZIS[4], G. MARÍN[2];
[1]Univ. de Chile, santiago, Chile; [3]Biología, [4]Sciences, Biol., [2]Univ. de Chile, Santiago, Chile; [5]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Mammals exhibit the highest degree of binocularity among vertebrates. However, the shape, orientation and extent of the binocular portion of the visual field is highly variable among them, ranging from a narrow, dorsal longitudinal band typical of lagomorphs and rodents, to the wide, frontal binocular field of primates. The extent and orientation of the binocular visual field shows a strong correlation with the visual ecology and behaviour of the animal; small prey animals, like rodents for instance, maintain a constant binocular overlap in the upper portion of the visual field during foraging, suggesting a role for this upper binocular space in anti-predatory surveillance and detection of suddenly appearing aversive stimuli. However, the developmental mechanisms that support the phylogenetic plasticity associated to these features remain largely unexplored. In this study we described the ontogeny of the visual field and orbit orientation in a diurnal precocial rodent endemic to Chile, *Octodon degus*. Degus are born eye-opened and display visually guided exploratory behaviours soon after birth. We performed visual field and orbit convergence measurements during the first postnatal month (P5, P9, P14 and P30) and in adults (at 6 - 12 months of age). As other rodents, adult degus displayed a longitudinal band of binocular field with a maximal azimuthal extension of ~60°. However, at P5 the maximal azimuthal extension reached only ~20° and progressively expanded at subsequent stages. Likewise, the angle formed between the frontal plane of each orbit progressively converged in parallel to the development of the binocular visual field. Along with these results, we described the course of development of the aversive responses to looming stimulation in the upper visual field. Notably, we found that the probability of escape to a looming stimulus increases progressively in parallel to the ontogenetic expansion of the binocular field. Our results reinforce the link between the aerial binocular portions of the visual field and reactive responses triggered by visual stimulation in rodents, revealing the strong dependence that exist between the maturation of morphological characters of the visual system and the behavior in which they participate.

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Poster

634. Visual Processing During Behavior

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Program #/Poster #: 634.02

Topic: D.06. Vision

Support: NIH Grant 5R01EY030156-02

Title: Measuring eye movement impairment in children with brain injury

Authors: ***S. MOONEY**, N. M. ALAM, G. T. PRUSKY;
Burke Neurolog. Inst., White Plains, NY

Abstract: Children with brain injury often exhibit cognitive or communicative deficits that impede traditional vision assessment, and eye movement impairments in such children can therefore often only be assessed with qualitative or low-resolution techniques. We used a novel vision test powered by eye tracking to determine whether spatial asymmetries in eye movements can be reliably quantified in children with brain injury. We measured saccade and pursuit eye movements in 69 children with and 14 children without brain injury (age 3 to 18) during an interactive game-like program called the Visual Ladder, in which the user pops randomly placed/moving virtual bubbles by fixating or tracking them. Mean saccade and pursuit distance for each child were binned into various opposing directional categories to analyze normative asymmetries and identify the children who deviated furthest from the line of best fit (>2 SD) in each comparison. Both healthy and brain-injured children exhibited significantly longer horizontal saccades/pursuits than vertical and longer downward saccades than upward (all $p < .001$; Fig. 1), in agreement with previously established biases. There were no other significant asymmetries. Children with brain injury did not have shorter saccades than healthy children, but did have significantly shorter pursuits ($p < .001$), which may be partially due to more fragmented pursuit detection from increased noise, and a wider spread for both saccades and pursuits. The 23 outliers detected across the six comparisons (red in Fig. 1) comprised 16 different children with brain injury, revealing how brain injury can manifest in distinct eye movement impairments. Our results demonstrate that the Visual Ladder can detect and quantify eye movement asymmetries in children with brain injury, including non-verbal children, and suggest that eye movements may be largely healthy in many such children. Further analysis will be needed to relate the distinct impairments apparent in our data to particular disease diagnoses and outcomes.

Disclosures: **S. Mooney:** None. **N.M. Alam:** None. **G.T. Prusky:** None.

Poster

634. Visual Processing During Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 634.03

Topic: D.06. Vision

Title: Different cognitive tasks elicit similar pupil responses

Authors: *H. G. FREY, A. R. JAGTAP, J. W. BRASCAMP;
Michigan State Univ., East Lansing, MI

Abstract: The pupil, known to be responsive to changes in luminance, also responds to changes in mental activity, with more cognitive effort correlated with a larger pupil dilation. Recent studies have used measurements of changing pupil size, pupillometry, as a noninvasive way to study cognition. However, there are a variety of mental tasks which require cognitive effort, and thus multiple ways to drive a pupil response. Despite being involved in different mental processes, the locus coeruleus (LC) and the superior colliculus (SC) are both associated with a pupil response when activated. We wondered if changes in pupil size caused by activation of different brain areas would yield distinct pupil signatures. If so, we might be able to obtain more detailed information about the cognitive task that produced the pupil response, such as which brain area was involved, in addition to just how much mental effort was expended. In this study we measured pupil area while participants were shown a random sequence of shapes. Each shape corresponded to one of three tasks: do nothing, increase or decrease a mental tally by 1, or shift spatial attention to a different sequence of the same shapes. The latter two cognitive tasks (alter tally and shift attention) were designed to involve processing with more emphasis on the LC and the SC respectively, and the "do nothing" task was included to identify which components of the pupil response were linked to the appearance of a target shape generally. We required participants to fixate on a dot at the center of the screen throughout the task. After discarding data with an excess of fixation breaks or incorrect trials, we analyzed the data from 57 participants. Using a deconvolution general linear model (GLM), we isolated event-related pupil responses for each of the tasks. We found that all three tasks resulted in a pupil dilation and had similar shapes and timing. While the main difference between task related responses was the amplitude of the response, this suggests an effect of differing levels of task difficulty rather than a task-specific signature. We also compared how the three curves depended on baseline pupil size – a known dependence in task-evoked pupil dilations – and found this dependence to be similar across the three tasks as well. In sum, we found no compelling differences in pupil responses between our three tasks – two designed to draw on quite distinct brain networks and a third a passive baseline task. Based on these findings, we conclude that pupil responses are strong indicators of cognitive effort regardless of the mental task, and that they do not seem to carry task-specific information in their signatures.

Disclosures: H.G. Frey: None. **A.R. Jagtap:** None. **J.W. Brascamp:** None.

Poster

634. Visual Processing During Behavior

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Topic: D.06. Vision

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Research to Prevent Blindness

Title: Cricket hunting as a behavioral assay for vision loss and rescue in mouse models of retinitis pigmentosa

Authors: *M. L. SCALABRINO[1], E. ZHANG[1], L. PETERS[1], M. PLUENNEKE[1], J. NOLT[1], M. THAPA[1], J. ROACH[2], S. S. X. LIM[2], J. WU[2], A. P. SAMPATH[3], J. CHEN[4], M. R. TADROSS[2], T. DUNN[2], G. D. FIELD[1];
[1]Neurobio., [2]Biomed. Engin., Duke Univ., Durham, NC; [3]Ophthalmology, UCLA, Los Angeles, CA; [4]Physiol. and Neurosci., USC, Los Angeles, CA

Abstract: Defining functional vision is essential to understanding how cell loss from inherited disease impacts the visual system, particularly to assay the effectiveness of gene therapy on pre-clinical models. Current behavior tasks for rodent models only capture certain aspects of vision, such as contrast sensitivity, or rely heavily on memory and other cognitive functions. Current behavior tasks also rely on artificial scenarios, such as forcing mice to swim, which introduces significant stress. An alternative behavioral approach would 1) capture a natural behavior, and 2) engage multiple aspects of visual processing.The cricket hunting behavioral assay satisfies both criteria. Mice primarily use vision to hunt and capture crickets in a laboratory setting (Hoy J et al., 2016). By modifying light levels, we isolated rod and cone contributions to functional vision in two mouse models of the inherited retinal disease Retinitis Pigmentosa (Cngb1neo/neo and rd10) over the course of degeneration. We also analyzed the impact of genetic rescue of vision loss on cricket hunting in the Cngb1neo/neo model. Time to capture was compared across groups either manually or using machine learning (3-Dimensional Aligned Neural Network for Computational Ethology, DANNCE, Dunn T et al. 2021). We correlated this behavioral data to (1) retinal physiology at matched timepoints using ex vivo multielectrode array measurements of retinal ganglion cell visual responses, and to (2) histological assessments of retinal synaptic structures. Manual estimates of cricket capture resulted in substantial variability compared to DANNCE estimates. Sighted mice under photopic illumination captured a cricket within one minute, comparable to other studies. Blind mice with total photoreceptor loss were unable to capture a cricket in under 3 minutes, equivalent to the performance of sighted mice in total darkness. Gene replacement improved scotopic capture times and cone-mediated photopic capture times were preserved for longer. These results correlate to retinal information rates calculated from retinal ganglion cell visual responses.Cricket hunting is a substantial improvement on current behavioral assays for functional vision in mice, given that visual deficits severely impede cricket capture and capture time can be determined using machine learning to

eliminate human variability in data analysis. Comparing this behavior to physiology and histology allows for a holistic understanding of the decline in vision in these preclinical models, as well as how gene therapy improves visual outcomes.

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Poster

634. Visual Processing During Behavior

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Ray Thomas Edwards Career Award

Title: Quadratic computations maintain neural specificity to natural stimuli across stages of visual processing

Authors: *R. ROWEKAMP, T. O. SHARPEE;
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Abstract: Biological visual systems achieve greater robustness of recognition compared to our most advanced artificial neural networks, which can make large errors in response to small perturbations. The computational motifs that underlie this robustness remain unclear. Here we analyze responses of neurons from the visual areas V1, V2, and V4 of the brain to find such motifs. Our analyses are based on a novel model architecture that extends standard convolutional networks by incorporating quadratic computations in order to capture nonlinear interactions between different visual features. The quadratic convolutional model achieved the same predictive power as leading machine learning methods. Being more interpretable, the quadratic convolutional model identified two computational motifs that were common across visual areas. First, at each stage either linear or quadratic computation dominated. Second, in cases with dominant quadratic computation, excitatory and suppressive features represented mutually exclusive features of visual stimuli, such as orthogonal orientations or opposing motion directions. Quadratic computations became progressively more important in subsequent brain areas. Quadratic computations at both local and global stages of the model increased neural

selectivity to natural stimuli. Overall, these results emphasize the importance of quadratic computations for achieving robust object recognition.

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Poster

634. Visual Processing During Behavior

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Topic: D.06. Vision

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Title: Effect of sleep on neural coding in early visual cortex revealed in a large population study

Authors: *M. ABDELHACK[1], P. ZHUKOVSKY[2,3], M. MILIC[1], D. FELSKY[1,4,5,6];
[1]Krembil Ctr. for Neuroinformatics, [2]Campbell Family Mental Hlth. Res. Inst., Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [3]Ctr. for Depression, Anxiety and Stress Res., McLean Hosp., Boston, MA; [4]Dept. of Psychiatry, [5]Inst. of Med. Sci., [6]Dept. of Biostatistics, Dalla Lana Sch. of Publ. Hlth., Univ. of Toronto, Toronto, ON, Canada

Abstract: Sleep is crucial for healthy brain function including the maintenance of memory, attention, and mood regulation functions. Recent evidence suggests bi-diretional relationships between sleep quality and mental health, using both self-report and objective accelerometry-based measures. However, the brain circuits that mediate these relationships are not known. Here we leverage a large sample of over 28,000 individuals with task-based and resting state fMRI, as well as accelerometry and self-report sleep data to identify the neural correlates of sleep quality and their relationships with cognitive function and mental illness in the general population. We found that visual coding impairment is associated with sleep in general visual tasks which relates to cognitive task performance. By analyzing visual task brain-wide fMRI data, we found an association between sleep deficiency extracted from accelerometry data and multivariate neural patterns in lower and intermediate visual cortex spanning both male and female individuals. These neural signatures and objective sleep measures were also related to cognitive abilities but not with self-reported mental health status or with self-reported sleep data. Conversely, the latter two were found to be correlated. Analysis of functional connectivity patterns using resting state brain data revealed a connection between the frontoparietal network and a visual network suggesting a reduction in top-down attention signaling as a possible cause for the visual processing impairment. Together, these results suggest a connection between sleep quality and visual cognitive processing in early visual areas, and is supported by previous work showing that sleep loss causes signal reduction in the visual cortex during working memory tasks. Our findings shed new light on the discrepancies between self-report and accelerometer-based sleep

quality measures and their unique relevance to depressive symptoms and cognition, as well as the role of visual processing impairment in moderating these relationships. This impairment could explain sleep-related variability in cognitive abilities and provide a novel target for non-invasive brain stimulation in sleep-deprived individuals.

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Poster

634. Visual Processing During Behavior

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Program #/Poster #: 634.07

Title: WITHDRAWN

Poster

634. Visual Processing During Behavior

Location: SDCC Halls B-H

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Topic: D.06. Vision

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2022m3e5e8017946

Title: The white gene-dependent ultraviolet avoidance in Drosophila

Authors: S. PARK[1,2], M. LEE[3,2], S. KIM[1,2], M. KIM[1,2], *K. KANG[4,1];
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Abstract: Although many beneficial effects of ultraviolet light to organisms have been reported, sensitively detecting and avoiding free radical-generating UV is important for animals because of its tissue-damaging and pro-aging effects. *Drosophila* are known to be photophilic to UV but their UV avoidance was reported to be weak, requiring preconditioning and prolonged illumination for proper avoidance. Here, we developed a simple and rapid assay testing UV locomotive avoidance using a handheld UV source that emits light peaking at 365 nm with natural intensities. This assay did not require preconditioning, but we found that the white gene is essential for the avoidance. The locomotive response to UV illumination begins with fast

attraction to UV source, and flies gradually moved away from it. Although UV avoidance is slower than attraction, the maximal avoidance was reached within two minutes. The w^{1118} mutant lacking the functional w gene did not show either robust attraction or avoidance. However, the mini *white* gene inserted in the genome with various transgenes did not efficiently rescue the defect, suggesting that the white gene expression is necessary in a very specific set of cells. Thus, our finding opens a new possibility for identifying components of machineries for UV avoidance.

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Poster

634. Visual Processing During Behavior

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Topic: D.06. Vision

Support: CIHR

Title: Spatial distribution of visual distortion in amblyopia

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Abstract: Amblyopia is clinically diagnosed by measuring visual acuity (VA), but amblyopes experience a pattern of visual deficits that may undermine VA measures — they experience visual distortions. These distortions are not simply spatial but include orientation and even spatial frequency distortions. Perceived visual distortions can simultaneously explain VA loss while being more powerful in explaining and characterizing the pattern of loss in individual patients. Several groups have shown that increased perceived monocular distortion is associated with poorer binocular function suggesting that monocular distortions are an impediment for binocular function. Thus, restoring binocular function requires capturing and eliminating visual distortions, something that VA and patching therapies do not attempt to address. In this study, we have therefore designed and tested a psychophysical task to fully capture amblyopic distortions across the central visual field with three distortion modalities (spatial Frequency, orientation and displacement distortions). Our protocol consists of multiple tasks, each testing one dimension of distortion by using a small array of stimuli to minimize crowding effects. The patients (all amblyopes) were shown two copies of the same array on two sides of the screen—one fixed, and one adjustable with a mouse/keyboard, and an eyepatch on a stick. They were trained to change the adjustable array such that it shows what their amblyopic distortion looks like. Once they felt they had captured their distortion, they advanced to a new trial where a new array was given. Over multiple trials, we accumulated information that allowed us to create a complete map of the distortions across visual space—one map per dimension. We were able to successfully and reliably capture the three components of visual distortions for each participant. Our results

suggest that all amblyopes in general perceive distortions that are non-uniformly distributed in their visual field. Furthermore, these maps exhibited a unique patterns of distortions for each participant. The intensity of distortions did not always correlate with the depth of amblyopia measured by the difference in VA between the amblyopic and the fellow eye. To our knowledge, this is the first study to have fully characterized perceived visual distortions in the amblyopic visual system. The transformative feature of our new stimuli design is that it yields separate maps distortions across the visual space. These individualized acquired maps have important clinical translation potential — they can be used as input for creating more fusible digital images to restore stereopsis in amblyopic patients.

Disclosures: **R. Abbas Farishta:** None. **H. Molaei:** None. **R. Farivar:** None.

Poster

634. Visual Processing During Behavior

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NIH EY014800
Research to Prevent Blindness

Title: Combining behavioral, physiological, and computational approaches demonstrates the neural basis of altered spatial perception during eye movements

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Abstract: The brain generates a perception of the visual world from complex spatial information in the environment. However, how neural activity in the visual cortex represents spatial information and how the visual perception of space is generated from this representation is unclear. To understand neurons' spatial coding, we examine how changes in location perception are linked to changes in neural activity within extrastriate areas. A known phenomenon involving altered spatial perception is perisaccadic mislocalization: the bias in the perceived location of visual stimuli appearing near the time of a saccade. This study uses a combined behavioral, physiological, and computational approach to establish a link between perisaccadic modulations in extrastriate responses and their behavioral counterparts during a mislocalization task in monkeys. We use array and single electrodes to simultaneously record neuronal activity in area V4 and the Frontal Eye Field (FEF) from sites with overlapping receptive fields (RFs). A monkey performs a visually guided saccade task while his eye movements are monitored with a

high-resolution eye tracking system. In each trial, the monkey makes a saccade from a fixation point to a peripheral saccade target. During fixation and saccade execution a 50-ms visual probe stimulus is presented in one of 9 possible locations in a 3x3 grid placed around the V4 neuron's RF. When the probe disappears, the monkey makes another saccade to the remembered location of the probe stimulus. Mislocalization is measured as the deviation between the reported location of stimuli presented around the first saccade to those presented during fixation. The neural recordings are used to fit an encoding model that characterizes the time-varying stimulus-response relationship over the course of the task with a high temporal precision. Beyond the precise description of V4 responses, the decoding aspect of the model allows us: 1) to develop a model-based readout of the visual scene, and 2) to selectively manipulate the model components, in ways that we cannot experimentally alter actual neural activity in the brain. This model-based readout and manipulation procedure enables us to isolate and assess the contribution of individual model components to alterations in the decoded location. Thus, our multifaceted approach offers a powerful tool for identifying specific V4 response components contributing to mislocalization, which will be validated by the experimental measurements. These analyses will reveal the neural substrate underlying the misrepresentation of space during eye movements, and the role of V4 and FEF neurons in the representation of location information.

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Poster

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Title: Behavioral states modulate the relationship between top-down input and inhibition within the mouse visual cortex

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Abstract: Sensory encoding within the visual cortex can shift dynamically based on an animal's behavioral state, which may serve to optimize encoding for distinct behavioral needs. Locomotion and pupil diameter (a measure of arousal) are two behavioral state spaces known to alter visual encoding. However, the signals that drive these state-dependent shifts in encoding are unclear. Top-down input from higher cortical areas, such as the anterior cingulate cortex (ACC),

can modulate information encoding in the visual cortex and likely contributes to these state-dependent shifts. The activity of a subset of inhibitory interneurons in the visual cortex, primarily VIP and NDNF positive interneurons, correlates with locomotion and pupil diameter; importantly, these neuron classes also receive input from ACC and other higher cortical areas and thus can mediate top-down influences. Indeed, their activity may represent a confluence of top-down modulation and state-dependent signals. However, these roles have largely been studied in isolation using different paradigms, and we have little understanding of how, for example, top-down signals in these interneurons are related to distinct behavioral states or vary across behavioral state transitions. To better understand these relationships, we leveraged dual-color in vivo calcium imaging to simultaneously record combinations of excitatory neurons, VIP interneurons, NDNF interneurons, and ACC axons within the mouse visual cortex as the mice viewed visual stimuli. We also simultaneously recorded locomotion and pupil diameter and delivered unexpected air puffs to evoke behavioral state shifts.
As expected, we found that excitatory visual responses and baseline excitatory population activity vary across behavioral states. VIP and NDNF interneurons both respond to visual stimuli and increase their activity in response to pupil dilation, but their time courses post-dilation diverge, with VIP activity remaining elevated longer than NDNF activity. VIP activity, but not NDNF activity, increases when the mouse begins running. ACC axons are visually responsive and selective, and these responses are modulated by running speed and pupil diameter. The different patterns and time courses of activity we observe in ACC axons and inhibitory interneurons across behavioral state transitions, and their correlations with changes in excitatory visual encoding, suggest that these signals coregulate state-dependent transitions within the visual cortex.

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Poster

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Title: The origins of Poisson spiking in the neocortex

Authors: *T. TAILLEFUMIER[1], J. J. PATTADKAL[2], D. H. BRAGER[3], N. J. PRIEBE[4];
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Abstract: The spiking responses of neocortical neurons are remarkably variable. Distinct patterns are observed when the same stimulus is presented in the sensory areas or when the same

action is executed in motor areas. This is quantified across trials by measuring the Fano factor of the neuronal spike counts, which is generally near 1, consistent with spiking times following a noisy Poisson process. The two candidate sources for noise are the synaptic drive that converges on individual neurons and intrinsic transducing processes within neurons. To parse the relative contributions of these noise sources, we made whole-cell intracellular recordings from cortical slices and used dynamics clamp to inject excitatory and inhibitory conductances recorded in vivo from visual cortical neurons (Tan et al. 2011). By controlling the conductance directly, we can test whether intrinsic processes contribute to poisson firing. We found that repeated injections of the same excitatory and inhibitory conductance evoked stereotypical spike trains, resulting in Fano factors near 0.2. Varying the amplitude of both excitatory and inhibitory conductances changed the firing rate of recorded neurons but not the Fano factor. These records indicate that intrinsic processes do not contribute substantially to the Poisson spiking of cortical cells. Next, to test whether differences in network input are responsible for Poisson spike patterns, we examined spike trains evoked by injecting excitatory and inhibitory conductances recorded from different presentations of the same visual stimulus. These records exhibited different behaviors depending on whether the injected conductances were from visually-driven or spontaneous epochs: during visually-driven epochs, spiking responses were Poisson (Fano factor near 1); during spontaneous epochs spiking responses were super-Poisson (fano factors above 1). Both of these observations are consistent with the quenching of variability by sensory stimulation or motor behavior (Churchland et al. 2010). We also found that excitatory conductances, in the absence of inhibition, are sufficient to generate spike trains with Poisson statistics. In summary, our results indicate that the Poisson spiking emerges not from intrinsic sources but from differences in the synaptic drive across trials, the nature of this synaptic drive can alter the nature of variability, and that that excitatory input alone is sufficient to generate Poisson spiking.

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Poster

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Title: Early cortical mechanisms of visual discomfort in premenarchal adolescents

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Abstract: Better understanding of the mechanisms underlying perceived unpleasantness in response to a stimulus are critically needed to improve the treatment of sensory disorders such as migraine and chronic pain. We previously found that generally healthy, young adult women with preclinical evidence of pelvic hypersensitivity reported greater visual unpleasantness than controls. Steady-state visually evoked potentials (SSVEPs) measured with scalp electroencephalography were associated with higher visual discomfort ratings, suggesting that early afferent activity in visual cortex may encode unpleasantness. However, other studies have obtained equivocal results. Assessing neural activity during visual stimulation prior to menarche—a time point where many sensory disorders become clinically apparent—may help eliminate potential confounders when exploring the relationship between neural activity and unpleasantness. Previously, we presented a blue-yellow checkerboard stimulus oscillating at 25Hz for 20 seconds across five increasing brightness intensities and gathered visual unpleasantness ratings following each trial block using a Gracely Box Scale (0-20). We demonstrated in a large cohort of adults (n=147) at greater risk for developing chronic pelvic pain (i.e., moderate-to-severe dysmenorrhea) that greater SSVEP amplitudes were associated with greater visual unpleasantness, even when adjusting for brightness intensity (at electrode Oz: partial eta-squared=.07, p_{fdr}=.02; fdr=false discovery rate). To establish whether visual hypersensitivity informs the development of sensory disorders, including postmenarchal pelvic pain conditions, we collected SSVEP data using the same procedure described above on a large cohort of premenarchal adolescents assigned female at birth (n=172). We replicated our EEG findings from the adults using linear mixed models. Specifically, visual unpleasantness ratings were predicted by 25Hz SSVEPs, even when adjusting for brightness intensity (occipital electrodes: R^2 betas=.001-.013, ps_{fdr}<.05). In contrast to the adults, the adolescents' topographies were more circumscribed to occipital and parietal sites with a right hemisphere bias. These results cross-validate our prior findings in a large cohort and further suggest that afferent activity in early visual cortex in part determines adolescents' subjective ratings of visual discomfort. Future directions of this work include identifying the role of visual hypersensitivity in predicting future sensory sensitivity and the development of pelvic pain.

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Poster

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Research to Prevent Blindness

Title: Methods for Decomposition of LFP Signals Within Visual Areas

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Abstract: Visual area V4 is known to be a crossroads for sensory, motor, and behavioral signals generating visual perception. Thus understanding the neural representation of visual information in this area requires dissociating and quantifying the contribution of multiple modulatory sources to V4 responses. Recent findings show that V4 neurons lock the timing of their spikes to the phase of certain V4 local field potential (LFP) oscillations, suggesting that a phase code may contribute to the neural representation in this area. However, reading a phase code is challenging since a measured LFP signal reflects the combination of multiple interfering oscillatory components received from local or distant cortical areas. Due to this interference, spike timing relative to the phase of a particular oscillation may be obscured when measured relative to the raw LFP recorded by an electrode. Therefore, resolving this oscillatory interference is necessary for accurately characterizing phase coding and its function in the V4 neural representation. This study focuses on separating the sources of oscillations driving an LFP signal using the blind source separation (BSS) methods. There are several existing approaches for the BSS problem such as independent component analysis, time-frequency analysis, linear transforms, adaptive techniques, matrix factorization, or neural network learning. Each of these methods introduces several assumptions and properties that may not be suitable for analyzing LFP data. This study investigates a data-driven signal decomposition framework that is applicable to LFP signals recorded simultaneously from multiple electrodes, where the decomposed sources are obtained based on a biologically plausible model describing the statistical features of the measured LFP patterns. We use this framework to decompose the LFP activity recorded along with the spiking activity of multiple V4 neurons using 16-channel Plexon linear array electrodes, while the monkey performs a visually guided saccade task with visual stimulation. The visual stimulus is composed of flashing visual probes with 7-ms temporal resolution that are presented around the receptive field area of V4 neurons. Decomposition of raw V4 LFP signals into their constituent oscillatory components will help us identify the modulatory sources generating V4 responses and measure a phase code relative to individual oscillatory components. Characterizing and tracing the dynamics of these sources over time will elucidate how phase coding can represent the visual information in area V4 and how the visual perception can be read out from this phase-based representation across saccadic eye movements.

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Poster

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Title: Natural scene expectation shapes the structure of trial-to-trial variability in mid-level visual cortex

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Abstract: What we expect to see can greatly affect what we perceive. How does expectation influence local circuitry among visual cortical neurons to allow for perceptual discrimination in the context of rich natural inputs? We have relatively little understanding of how populations of sensory cortical neurons change their responses with expectation, as the majority of studies manipulate expectation in a task-irrelevant way, disallowing for comparisons of changes in neuronal population activity with behavioral performance. Theoretical and experimental work indicates that changes at the population level, in particular those related to the variability in neural responses, greatly impact stimulus encoding. Therefore, we sought to investigate whether changes in the structure of neural population activity in mid-level visual cortex might underlie the behavioral advantages conferred by forming an expectation. We recorded from populations of visual cortical area V4 neurons using implanted 96-electrode "Utah" arrays in macaques engaged in a natural scene change detection task in which we modulated image expectation. During high expectation blocks, the same image (e.g. image A) was used on every trial. During low expectation blocks, a random image (from 10 possible images, including image A) was chosen for each trial, thereby reducing the expectation that image A would be seen on a given trial. Comparisons were made for image A during high and low expectation blocks. New natural scenes were selected for each session. Data from 2 monkeys showed a robust improvement in behavioral performance (elevated d-prime) when the image was expected. Our recordings showed that expectation decreased neuronal responses and modulated short timescale (within-trial) adaptation. Using dimensionality reduction methods (factor analysis), we found a decrease in shared variability which was correlated with increased behavioral performance. In summary, we have developed a task that allows us to assess the impact of expectation on neuronal activity when expectation is relevant to the subject's success, enabling us to pair neuronal findings with behavioral outcomes. Our findings suggest that shifts in individual neuron activity and changes in shared variability underlie perceptual improvements due to expectation, offering a novel view into the different ways in which cognitive processes can affect sensory areas. Our results support the idea that expectation is built through the interactions among populations of neurons relatively early in the visual system, enabling it to be flexible for arbitrary visual scenes and objects.

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Poster

634. Visual Processing During Behavior

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Topic: D.06. Vision

Title: State dependency of neuronal variability

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Abstract: Despite large variability in spiking responses, neuronal populations robustly encode stimulus information leading many to speculate on the role of spiking variability. One way to investigate the conundrum is to partition variability into different origins. Previous work suggests that the sources of neuronal variability primarily derive from three categories: independent stochastic processes, brain state modulation, and external environmental influence. In this work, we systematically analyzed the influence of brain states on spiking variability in the visual cortex using large-scale recordings from the Allen Brain Observatory dataset. Specifically, we define brain states based on frequency-specific transients in local field potentials (LFP), long shown to correlate with different cognitive functions. To alleviate random noise in the oscillations, we redefine LFPs as a point process of transient bursts, separately, in the theta (3-8 Hz), beta (10-30 Hz), lower gamma (30-50 Hz), and higher gamma (50-80 Hz) frequency ranges. Using hidden Markov modeling, we identify global brain states from patterns of local power fluctuations in the LFP events. Across field potential recordings from all visual areas, the model identified three states, a high-gamma state, a high-theta state, and an intermediate state with a moderate theta to gamma ratio. Analysis of spiking response to repeated natural movie presentations showed that population spiking patterns had lower pairwise cosine similarity and higher mutual information (MI) with visual stimuli (movie frames) during high-gamma states. Single neurons, in these states, reported lower Fano factor values and lower percentages of shared variance. High-gamma states were also associated with higher running speeds and larger pupil sizes. All the above trends were reversed during high-theta states. Recent work on metastable attractor systems has suggested the role of variability in influencing state transitions. In the visual cortex, we found that individual neurons showed the highest precision and reliability to drifting gratings, followed by the frame-shuffled movie and natural movie presentations. To that end, we analyzed the state transition patterns during the different stimulus presentations. State changes occurred less frequently during movie presentations, with longer durations spent in the low-arousal/high-theta state. By contrast, drifting gratings induced more frequent state changes, yet, subjects spent longer durations in high-gamma states than in other stimuli. Overall, our results support the premise that neural variability plays a vital role in affecting states and state transitions.

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Poster

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Research to Prevent Blindness

Title: The contribution of a neural phase code to sensory encoding during eye movements

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Abstract: The planning and execution of eye movements induce strong oscillations in local field potentials (LFPs) within visual cortical areas. Whether these oscillations contribute to the enhancement of sensory representations during eye movements is not known. In order to test this idea, we recorded LFPs and spiking activity simultaneously from both area V4 and the Frontal Eye Field (FEF) in macaque monkeys. The animal performed a visually guided saccade task. During the task, a sequence of small visual probes was presented on the screen at different locations in the visual field, before, during and after the execution of the eye movement. We found a strong locking between the time of spikes and the phase of LFPs, particularly in the 4-35 Hz frequency range. However, the V4 preferred phase, i.e., the phase at which most of the spikes occur, systematically changed around the time of eye movements. This resulted in robust spike-phase locking only in shorter temporal windows. However, the variation in preferred phase deteriorated our ability to develop a consistent readout based on the phase of spikes in larger time windows. Interestingly, V4 spikes also showed similar variation in their preferred phase relative to the FEF LFP. The temporal co-occurrence of the oscillatory signals in FEF and V4 inspired us to use the LFP signal of area FEF to decompose the V4 raw oscillation into two components, one shared with FEF and one exclusive to V4. Using the V4-exclusive oscillations we found that the preferred phase of V4 spikes stayed comparatively stable across eye movements. Moreover, different probe locations evoked spikes at different phases. Whereas using the raw V4 LFP, discrimination of probe locations based on the phase of spikes was poor, using the V4-exclusive oscillation, the location information was traceable across eye movements using the spike phase information. This revealed the capacity of a phase code for the representation of spatial information during the fast dynamics of eye movements. LFPs are believed to be the result of a combination of multiple local and distant oscillatory sources. These results highlight the importance of isolating individual oscillatory components for a reliable readout of information based on a phase code.

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Poster

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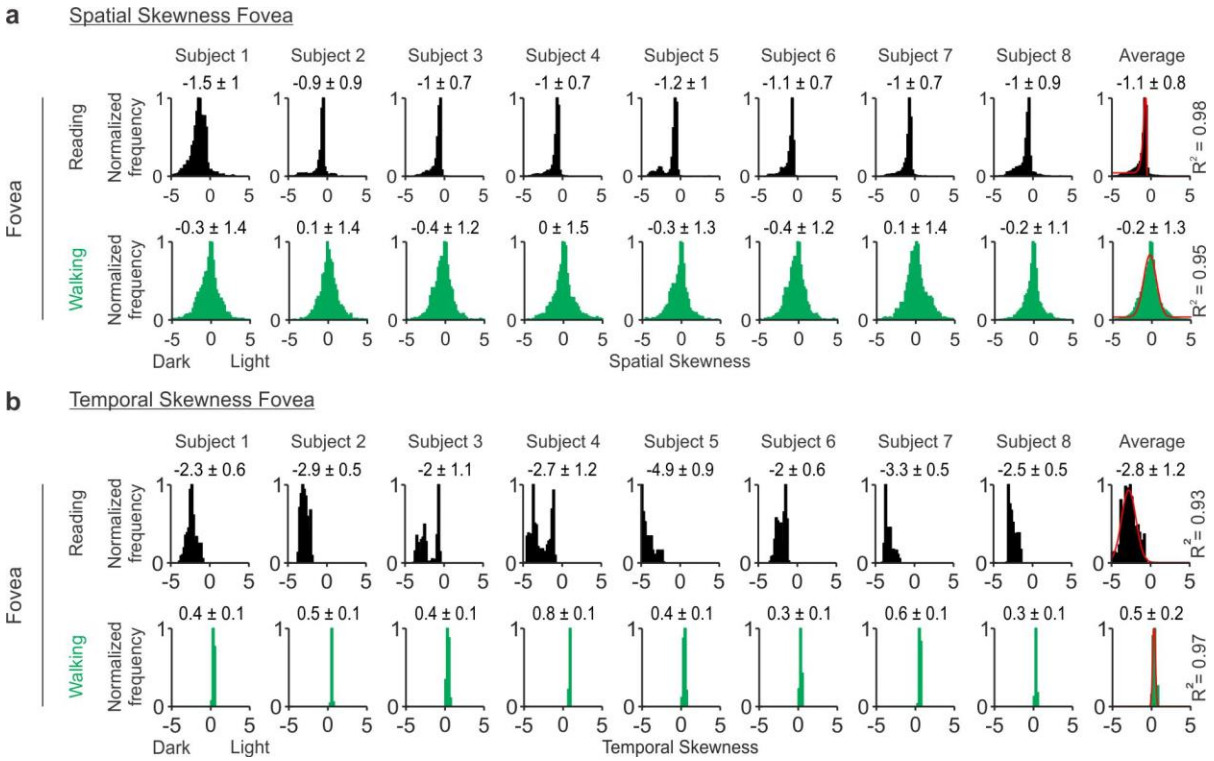
Title: Visual navigation equalizes retinal stimulation of ON and OFF pathways

Authors: *S. POUDEL, H. RAHIMI-NASRABADI, J. JIN, S. NAJAFIAN, J. M. ALONSO; Dept. of Biol. Sci., State Univ. of New York , Col. of Optometry, New York, NY

Abstract: ON and OFF visual pathways respond to stimuli with different contrast polarity and spatiotemporal properties. Because stimulus properties are continuously changing in our visual worlds, the stimulation balance of ON and OFF visual pathways should also be changing. Here, we investigate how the stimulation balance of ON and OFF pathways changes during visual navigation (walking) when compared with stationary activities (reading). We compared the visual input feeding the retina of human subjects (2 females, 6 males, 29.6 ± 6.1 years old) performing these two tasks indoors while wearing glasses with cameras and sensors that recorded visual images and visuomotor activity (Tobii Pro Glasses 2). To quantify the visual input, we measured the spatial and temporal skewness of the visual scene at the fovea, defined as the central portion of the scene around the fixation point (5 degrees of diameter). The spatial and temporal skewness were calculated as the normalized average difference between each pixel luminance and their mean, measured across space (spatial) or time (temporal). Our results demonstrate that walking generates images at the fovea that have much more balanced light/dark contrast (i.e. skewness closer to zero) than reading. The equalization in light/dark stimulation balance could be demonstrated with measurements of spatial skewness (5.5 times increase in light/dark balance, -0.2 ± 1.3 for walking, -1.1 ± 0.8 for reading, $p < 0.0001$, Wilcoxon test) and temporal skewness (6.6 times increase in light/dark balance, 0.5 ± 0.2 for walking, -2.8 ± 1.2 for reading, $p < 0.0001$, Wilcoxon test). Moreover, the variation in temporal skewness across space was 6 times more restricted during walking than reading (0.2 vs. 1.2 standard deviations) and all measurements were very consistent across subjects (Figure 1). Based on these findings, we conclude that visual navigation equalizes the light/dark contrast of the images projected at the fovea across both space and time and, by doing so, it increases the stimulation balance of ON and OFF visual pathways.



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Poster

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Title: Running modulates primate and rodent visual cortex via common mechanism but quantitatively distinct implementation

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Abstract: When mice actively locomote, visual signals in their primary visual cortex (V1) are strongly modulated. This observation has fundamentally altered conceptions of a brain region previously assumed to be a passive image processor, and extensive work has followed to dissect the sources, recipients, and functional consequences of running-correlated modulation. However, it remains unclear whether visual processing in primates might similarly change during active locomotion. We therefore measured V1 activity in a nonhuman primate, the common marmoset (Callithrix jacchus), while they alternated between running and stationary. In contrast to the large increases in mouse V1 during running, conventional metrics of response in marmoset V1 were slightly but reliably decreased during running. However, by leveraging large-scale recordings, analysis of the latent variables driving population activity revealed a common mechanism in both species: trial-to-trial fluctuations of shared gain modulations were present across V1 in mice and marmosets. These gain modulations were larger in mice and were often positively correlated with running; they were smaller and more likely to be weakly negatively correlated with running in marmosets. Thus, population-scale gain fluctuations of V1 reflect a common principle of mammalian visual cortical function, but there are important quantitative differences in their magnitudes and correlations with behavior that yield distinct consequences for the relation between vision and action in primates versus rodents.

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Poster

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AFOSR 19RT0316

Title: Modulation of V1 neurons by visual input and motion in freely-moving marmosets

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Cortical Systems and Behavior Laboratory, Dept. of Psychology, UCSD, San Diego, CA

Abstract: Historically, studies of visual cortex have been performed while nonhuman primates are head-fixed and viewing visual stimuli on a screen. This approach, however, is divorced from one of the primary factors that has driven the evolution of brains, including sensory systems, namely the ability to move through the world. In the real world, visual processing must accommodate how we actively explore the environment while making choices about when and where to go. Despite its clear significance, virtually nothing is known about how the primate visual system supports natural, active vision in freely moving animals. To address this problem, we leveraged an innovative, head-mounted eye-tracking system developed for marmosets in our

lab while simultaneously recording the activity of single neurons in primate V1 to quantify the degree to which multiple sources of motion - eye, head, body - and visual input modulated neural activity. In these experiments, monkeys are first head-fixed to perform eye calibration and shown traditional visual stimuli known to modulate primate V1 neurons. Next, the monkey is carefully removed from the chair without the recording being paused, and placed in the arena where similar visual stimuli are shown on the floor and wall. With the data collected, we aim to 1) calculate receptive fields and tuning curves in V1 neurons under freely moving circumstance; 2) compare the receptive fields and tuning curves in V1 neurons between the head-fixed and freely moving scenarios; 3) quantify the effects of eye, head and body movement on the primary visual cortex activity. Our work is the first attempt where the monkey is completely unconstrained, and the modulation of different aspects along with visual stimuli are combined in understanding visual processing in primary visual cortex.

Disclosures: **J. Li:** None. **V.P. Singh:** None. **C.T. Miller:** None.

Poster

634. Visual Processing During Behavior

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Topic: D.06. Vision

Support: NIH UF1 NS116377
AFOSR 19RT0316

Title: Embedded system-based neurophysiology coupled head mounted eye tracking in freely moving marmosets

Authors: *V. SINGH, J. LI, C. MILLER;
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Abstract: Brain activity and computation varies as a function of environmental cues/interactions. There is no guarantee that results from highly controlled sensory deprived experiments will translate to more dynamic and complex freely moving behaviors. In the past, most of the vision neuroscience experiments in non-human primates were performed in head restrained conditions and we have very limited understanding of how the eye behavior and brain states change when animals are truly freely moving. This is mostly a result of lack of reliable ambulatory eye tracking systems. In our current study, we demonstrate an embedded system-based camera system called CEREBRO (Chair-free Eye Recording using Backpack mounted Microcontrollers) to perform reliable eye tracking in freely moving common marmosets. Eye tracking synchronized with a front facing scene camera (also part of the same system) gives us a good estimate of what is falling on the animal's retina during a very complex active exploration in an open arena. We track their head and body movements (using motion tracking cameras) while simultaneously performing brain electrophysiological recordings (using a microwire brush

array) in a wake freely behaving animal. We found that brain state changes drastically between head fixed and head free conditions for the same session and that the majority of eye movements are compensatory forehead movements which stabilize the visual scene. Unlike classical eye-tracking, we do not have much control on the illumination of the eye and image contrast parameters. Therefore, we developed a novel eye tracking pipeline that uses a segmentation artificial Neural network to perform pupil detection. This method will provide a platform for studying the perceptual and neural basis of active vision during ethological behaviors in non-human primates.

Disclosures: V. Singh: None. **J. Li:** None. **C. Miller:** None.

Poster

634. Visual Processing During Behavior

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Topic: D.06. Vision

Support: The Stratton VA Medical Center
P41-EB018783 (NIH, NIBIB)
NYS Spinal Cord Injury Research Board
Buchanan Fellowship
Catalyst Grant

Title: Validating an SSVEP-based BCI for people with locked-in syndrome

Authors: *P. KEERTHI[1,2], E. HITCHCOCK[3], C. FRANZ[3], T. M. VAUGHAN[1], H. HABIBZADEH[1], J. J. NORTON[1];
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Abstract: The MusicBox (MB) is a Brain-Computer Interface (BCI) controlled by steady-state visual evoked potentials (SSVEPs) that provides music as feedback. The purpose of this study is to explore the potential for reliable and sustained MB control for a person with locked-in syndrome resulting from amyotrophic lateral sclerosis (ALS) (S5). He now uses the MB at home, but improved control could provide him with Yes/No communication. The MB elicits an SSVEP by alternating between light and dark gray squares at 7.5 Hz (PsychToolBox) and records and processes SSVEPs for the first 6 harmonic sinusoids of the stimulation frequency from 8 scalp locations (Fz, Cz, P3, Pz, P4, Po7, Po8. Oz) using BCI2000 and canonical correlation analysis (CCA). The participant receives visual feedback from a semi-transparent overlay whose size is directly proportional to the real-time maximum canonical correlation (MCC) of the user's SSVEP. When the MCC reaches a preset threshold, a transparent overlay changes color and music plays. Four neurotypical participants (S1-4, 3 males, 19-62yr) and one with ALS (S5, male, 46yr, 0 ALSFRS) gave informed consent and completed between 40 and 60 30s trials with

the goal of playing music continuously. To establish that S5 had an appropriate SSVEP response, we examined good (top 10% of MCC scores) and bad (bottom 10%) trials for each participant. We used the area under the precision-recall curve (AUC-PRC) to quantify performance of binary classification (stimulus on or off) based on a thresholded MCC. (AUC-PRC S1-S4 top 10%: 0.945 ± 0.001 (SEM), bottom 10%: 0.899 ± 0.003; S5 top 10%: 0.914 ± 0.010, bottom 10%: 0.681 ± 0.018). An AUC-PRC of .5 indicates chance. To quantify how long each state is correctly detected without incorrect transitions, we calculated the maximum M value produced at an optimal threshold described by Habibzadeh et al. (IEEE ICASSP, 2021) (M=1 signifies perfect state detection). The mean M value for S5 was lower in both good and bad trials (top 10%: 0.458 ± 0.035, bottom 10%: 0.172 ± 0.009) compared to healthy controls (top 10%: 0.834 ± 0.011, bottom 10%: 0.485 ± 0.008). The high AUC-PRC in the top 10% of trials compared to low AUC-PRC in the bottom 10% of trials indicates a present but inconsistent SSVEP in a person with locked-in syndrome due to ALS. Participant S5's comparatively low M values indicate additional challenges, for example, maintaining MB control may require sustained attention. In the future, we will test how the use of improved algorithms may refine MB control of SSVEP-based BCIs for communication by people with severe motor disabilities.

Disclosures: **P. Keerthi:** None. **E. Hitchcock:** None. **C. Franz:** None. **T.M. Vaughan:** None. **H. Habibzadeh:** None. **J.J. Norton:** None.

Poster

634. Visual Processing During Behavior

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Support: Stratton VA Medical Center
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NYS Spinal Cord Injury Research Board

Title: Optimizing stimulation frequency for brain-computer interface-based detection and definition of color vision deficiencies

Authors: A. ATKINS[1,2], H. HABIBZADEH[1,3], K. J. LONG[1], T. M. VAUGHAN[1], ***J. NORTON**[1,3];
[1]Natl. Ctr. for Adaptive Neurotechnologies, US Dept. of Veterans Affairs, Albany, NY; [2]Neurosci., Union Col., Schenectady, NY; [3]Electrical and Computer Engin., Univ. at Albany - State Univ. of New York, Albany, NY

Abstract: More than 20% of people worldwide are affected by congenital and acquired color vision deficiencies (CVDs). Recently, we demonstrated a new brain-computer interface (BCI)-based method for detecting CVDs that has several advantages over existing methods; it is fully automatic, does not require the user's active participation, and may provide a more precise

diagnosis of CVDs. This method measures steady-state visual evoked potentials (SSVEPs) elicited by alternating light sources that differ in hue or luminance. Our initial studies used 10 Hz stimulation to elicit SSVEPs. However, previous research shows that SSVEPs elicited by low frequency stimulation (i.e., 0.5-5 Hz) are more sensitive to hue-based differences between light sources, while SSVEPs elicited by higher frequency stimulation (i.e., 10-50 Hz) are more sensitive to luminance-based differences between light sources. Thus, we hypothesized that BCI-based detection and definition of CVDs may be improved by optimizing stimulation frequency. To test this, we performed experiments with 3 participants to measure SSVEPs elicited in response to 4 classes of stimuli: (1) stimuli with different hue/equal luminance; (2) stimuli with different luminance/equal hue; (3) a stimulus with equal hue/equal luminance but different spectra (i.e., a metamer); and (4) a stimulus that alternated between on and off. For each class of stimuli, we measured SSVEPs (using EEG) elicited by light sources alternating at 9 distinct frequencies between 2 and 38 Hz. Participants completed ≥ 4 runs of data collection; each run contained 90 trials. We measured the size of the SSVEP elicited during each trial using canonical correlation analysis. The stimulus with equal hue/equal luminance elicited the smallest SSVEPs (max $r = 0.51$), and the stimulus that alternated between on and off elicited the largest SSVEPs (max $r = 0.85$ at 12 Hz). Baselined to the stimulus with equal hue/equal luminance, SSVEPs elicited by stimuli with different hue/equal luminance were largest between 4 and 10 Hz, whereas for stimuli with different luminance/equal hue, the differences were largest between 12 and 20 Hz. To identify the optimal stimulation frequency, we performed hypothesis tests that assessed the statistical significance of differences between the SSVEPs elicited by the stimulus with equal hue/equal luminance and the other 3 classes of stimuli. The differences were most significant (i.e., stimulation was optimal) at 16 Hz ($p = 0.00046$). Thus, the use of 16 Hz stimulation or specific stimulation frequencies to assess hue (4-10 Hz) vs. luminance (10-20 Hz) differences between light sources may improve BCI-based detection and definition of CVDs.

Disclosures: **A. Atkins:** None. **H. Habibzadeh:** None. **K.J. Long:** None. **T.M. Vaughan:** None. **J. Norton:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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Topic: D.06. Vision

Support: NIMH project #ZIAMH 002032
KAKENHI (19K12149; 20K12588; 22K12189; 19J40302)
New Energy and Industrial Technology Development Organization (NEDO)

Title: Neural activity in area TE, not TEO, correlates with monkeys' learning in a visual categorization task

Authors: *W. WANG[1], B. LI[2], M. A. ELDRIDGE[3], N. MATSUMOTO[5], K. HAYASHI[5], K. MARTINEZ GOMEZ[6], Y. SUGASE-MIYAMOTO[5], B. J. RICHMOND[4];
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Abstract: Primates, including old-world monkeys, can categorize images quickly based on similarity of visual features. We previously showed that two subregions of inferior temporal cortex, areas TEO and TE, contribute to visual categorization to differing extents. Monkeys with TE removals show a more sustained deficit than monkeys with TEO removals in the categorization of trial-unique images. The neural mechanisms underlying these differences are still unclear. Here we recorded simultaneously from TE and TEO using implanted 96- or 64-channel Utah arrays while two monkeys were learning a visual categorization task (morphed cats and dogs) after they had learned the task rules with trial-unique images (nonmorphed cats and dogs). Using a shallow neural network for population-level decoding, we found that decoding accuracy of TE neurons increases monotonically during learning, approximately paralleling the monkeys' behavior. In TEO, decoding accuracy increased on the second day, but showed no further improvement on subsequent days. At the single neuron level, neurons in TE, not TEO, are increasingly modulated by image category during learning. The variance in neural activity explained by image category increases monotonically for TE neurons, not TEO neurons. Furthermore, ROC (Receiver operating characteristic) analysis shows that in single neurons, encoding of the image category associated with larger reward is enhanced in TE, not TEO, during learning. These results indicate that neural activity in TE, not TEO, correlates with learning of visual categories.

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Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 635.02

Topic: D.06. Vision

Support: NIMH project #ZIAMH 002032

Title: Dissociation of function of visual areas TEO and TE in tests of visual recognition memory and perceptual categorization

Authors: *M. A. ELDRIDGE[1], J. E. PEARL[2], T. SETOGAWA[3], B. J. RICHMOND[1];
[1]NIMH, NIMH, Bethesda, MD; [2]Harvard Univ., Cambridge, MA; [3]Toyama Univ., Toyama, Japan

Abstract: It is widely accepted that a set of interconnected cortical brain regions running from the occipital pole to the ventral temporal lobe underlie the ability to perceive and recall visual stimuli.This pathway, labeled the 'ventral visual stream', consists of architectonic areas starting with primary visual cortex (V1) in the occipital lobe through V2, to V4, to the inferior temporal cortex areas TEO and TE. Although it has been established that there are reciprocal anatomical connections between areas, this system is often modeled as a multi-layer, feed-forward network (a deep network). We performed two experiments to study the contributions of two areas thought to be at the final stages of complex image processing, areas TEO and TE. In a visual perceptual categorization task (the monkeys had to judge whether a morphed image was more dog-like or cat-like) bilateral removals of either area TEO or area TE led to a modest impairment in performance, and when both TEO and TE were removed, the deficit was severe. Thus, the two regions seemed to contribute to the ability to categorize stimuli accurately, and each could at least partially compensate for the loss of the other. Conversley, in a visual recognition task (the monkeys had to indicate whether a series of novel images were being presented for the first or second time within a session), TEO removal had no effect on performance, whereas TE removal caused a severe impairment. The combined TE + TEO removal produced no additional deficit to that observed in the group with TE removals. Both of these results demonstrate that TE receives high resolution visual information even when TEO has been removed. Further, the dissociation in the pattern of effects observed in these two studies demonstrates specialization of function in areas TE and TEO.

Disclosures: **M.A. Eldridge:** None. **J.E. Pearl:** None. **T. Setogawa:** None. **B.J. Richmond:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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NRF-2019R1A2C1085566

Title: Spin-echo BOLD fMRI provides high specificity for cortical layer-dependent representations in human primary visual cortex

Authors: *R. KIM[1,2], S. HAN[1], W. SHIM[1,2,3];
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Abstract: Recently, ultra-high field (UHF) fMRI enabled us to investigate the mesoscopic regime of cortical layers and columns. By taking advantage of UHF, laminar fMRI with submillimeter resolution gives us an opportunity to examine layer-dependent neural activity such as feedforward and feedback processes. Conventionally, the gradient-echo (GE) blood oxygenation level-dependent (BOLD) signal has been widely used to examine fMRI, but its spatial specificity is low due to the sensitivity to large draining veins at the cortical surface. On the other hand, spin-echo (SE) BOLD is only sensitive to small vessels close to the location of neural activity, which leads to high spatial specificity. Here, we used GE-BOLD and SE-BOLD to compare layer-dependent visual representations. We acquired both signals together in the same repetition time while participants viewed apparent motion (AM) stimuli. The AM stimuli consisted of two alternatively presented Gabors whose orientations were orthogonal to each other. Regions of interest (ROIs) corresponding to the location of the stimuli and the mid-point between the stimuli were localized in the primary visual cortex, which contains stimulus- and internally-driven neural signals representing physically presented and interpolated orientation (Chong et al., 2016), respectively. In stimulus-driven ROIs, the GE-BOLD signal increased toward superficial layers, which reflects the effect of draining veins. In contrast, the SE-BOLD signal showed its peak activation in the middle layers where stimulus-driven feedforward signals are dominant. Using an orientation encoding model (Brouwer and Heeger, 2009), we also reconstructed the orientation represented in each layer and calculated the difference between the actual and reconstructed orientation. We found that SE-BOLD outperformed GE-BOLD in terms of precision in reconstructed orientation. In particular, SE-BOLD revealed more precise orientation representation in the middle layer compared to superficial and deep layers, whereas GE-BOLD did not show significant differences across layers. Similarly, in internally-driven ROIs, the spatial specificity was higher for SE-BOLD compared to GE-BOLD regarding both activation profiles and orientation encoding results. Notably, SE-BOLD revealed distinct layer-dependent patterns of response between stimulus- and internally-driven ROIs, which suggests that the sources of the neural signal are different for stimulus- and internally-driven visual representation. In sum, SE-BOLD offers high spatial specificity across cortical layers, which provides an opportunity to investigate layer-dependent visual information.

Disclosures: **R. Kim:** None. **S. Han:** None. **W. Shim:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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Title: Primate V2 receptive fields derived from orientation tuning and retinotopy of V1 inputs

Authors: *M. S. HASSANPOUR[1], S. MERLIN[1,2], F. FEDERER[1], Q. ZAIDI[3], A. ANGELUCCI[1];
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Abstract: In the primate visual cortex, object recognition occurs via a series of transformations through hierarchically organized areas in the ventral visual pathway. Along this hierarchy, neuronal receptive fields (RFs) become tuned to progressively more complex image features, e,g. V1 cells are tuned to the orientation of line segments (Hubel & Wiesel, 1968) while V2 cells respond to more complex contours, shapes and textures (Hedge & Van Essen, 2000; Freeman et al 2013; Heydt et al 2000). The circuit mechanisms and computations that mediate the increasing complexity of RFs along the cortical hierarchy are unknown as we lack knowledge of the fine-scale connectivity rules between areas. Here, we combined anatomical labeling and functional imaging to investigate the functional organization of V1 inputs to V2 and developed a computational model to investigate how these inputs contribute to generating V2 RFs. Orientation and retinotopic maps were recorded in V1 and V2 using intrinsic signal optical imaging and retrograde tracers were injected into single orientation columns in V2 (n=8 injections in 3 macaque monkeys). Confocal images of V2 injection sites and V1 labeled cells were aligned to the functional maps and each labeled cell in V1 and pixel at the V2 injection site was assigned a RF retinotopic location and preferred orientation based on its position on the maps. We found that, although the majority of V1 neurons projecting to a V2 orientation column had similar orientation preference to that of their V2 target column, V1 inputs showed a spatial spread in RF centers and a diversity of preferred orientations, forming complex visual patterns. We next modeled the spatial V2 RFs at the injection site as a linear combination of their V1 inputs, the latter in turn modeled as Gabor filters with parameters estimated from measurements of RF position and orientation tuning. Weights in the linear model were estimated for each V1-V2 cell-pixel pair as the dot product of their mean-zeroed tuning curves. Modeled V2 RFs could be roughly classified as elongated V1-like filters, or filters with relatively complex spatial structures. The responses of modeled V2 filters to grating stimuli closely resembled measured V2 responses. When presented with a large (>70,000) set of natural image patches, compared to V1 filters, V2 filters preferred a different subset of natural image patches containing more complex features such as elongated contours, angles, textures, and texture-defined borders.Our results suggest that converging V1 inputs can account for the more complex RFs of V2 neurons according to a simple combination rule.

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Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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Topic: D.06. Vision

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Title: Interhemispheric projections from ventral visual areas to areas TE and TEO

Authors: *B. LI, K. MARTINEZ GOMEZ, N. MIYAZAKI, M. A. ELDRIDGE, W. LERCHNER, B. J. RICHMOND;
NIMH, Bethesda, MD

Abstract: Visual information is integrated along the hierarchical areas of ventral visual pathway. The receptive field (RF) of visual neurons gradually increases from V1, V2, V4 to inferotemporal cortex (IT). In the two subdivisions of IT, areas TE and TEO, the RFs of visual neurons extend across the midline, thus including both contralateral and ipsilateral visual field representations. Previous studies reported that the representations of the ipsilateral visual fields of IT neurons depend on forebrain commissures. Combined transection of anterior commissure and splenium of the corpus collosum eliminates the visual response in the ipsilateral visual field. To study the source of the ipsilateral visual information, specifically which types of projections could contribute - feedforward, feedback, or contralateral - we injected viruses that induce retrograde transport of their gene products (FuGE, 8 sites x 20uL per site, 2*10^9 IU/ml) unilaterally into areas TE and TEO of two rhesus monkeys. Area TE received strong interhemispheric projections from contralateral TE, and weaker projections from contralateral TEO. Both sets of projections were mainly from layer III. The projections from contralateral V4 were very sparse. Area TEO received interhemispheric projections mostly from contralateral TEO. It also received significant projections from contralateral V2 and contralateral V4. The contralateral projecting neurons from TEO, V4 and V2 were located almost entirely in layer III. There were also sparse projections from contralateral TE to TEO, again, mainly originating in layer III. These results suggest that area TE receives visual information about the ipsilateral visual field at the same hierarchical level, i.e., from area TE in the other hemisphere. In contrast, area TEO not only receives ipsilateral visual information from the other side of TEO, but also from feedforward projections of contralateral V2 and V4.

Disclosures: B. Li: None. **K. Martinez Gomez:** None. **N. Miyazaki:** None. **M.A. Eldridge:** None. **W. Lerchner:** None. **B.J. Richmond:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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Program #/Poster #: 635.06

Topic: D.06. Vision

Support: NIMH project #ZIAMH 002032

Title: Retrograde viral tracing demonstrates bifurcating V4 projections to areas TEO and TE in the rhesus monkey

Authors: *K. MARTINEZ GOMEZ, B. LI, N. MIYAZAKI, M. A. ELDRIDGE, W. LERCHNER, B. J. RICHMOND;
Lab. of Neuropsychology, NIMH, Bethesda, MD

Abstract: The classic hierarchical structure of ventral visual pathway (V1 -> V2 -> V4 -> TEO -> TE) posits that visual information reaches area TE via area TEO. However, a behavioral study using selective lesions from our lab suggests that visual categorization can be performed in areas TEO and TE in parallel. Previous anatomical studies show that a direct projection from V4 to area TE exists, besides the V4-TEO-TE pathway. To study whether the V4->TE projection could be large enough to support high-resolution visual behavior (categorize cats vs dogs), we injected nonreplicating lentiviruses psuedotyped with the rabies coat protein (FuGE) in two rhesus monkeys. This configuration of the virus leads to retrograde transport of gene product from axon terminals throughout the neuron. In monkey S, we injected FuGE viruses expressing fluorescent reporter GFP into area TEO, and mCherry into area TE. In monkey D, the viruses used were switched between areas TEO and TE. The viruses (8 x 20 uL, at a titer of $2*10^9$ IU/ml) were delivered using a multi-channel array injector to cover a broad area in both TEO and TE. We found TE-projecting and TEO-projecting neurons in V4, V4v, and V4t in both monkeys, which covered V4 areas representing both central and peripheral visual fields, but the density varied, i.e., TEO received more projections from V4v and Vt than did TE. TE-projecting and TEO-projecting neurons were intermixed across the areas V4 and V4t but were in separate clusters in V4v. The results were generally consistent across monkeys, with one exception: V4 showed fewer projections to TE in monkey D than monkey S. This might be because of the injection site differences, i.e., the TE injection was more rostral in monkey D than in monkey S. In addition, we found sparse populations of neurons with bifurcated projections to areas TEO and TE, in V4, V4v, and V4t of both monkeys. These results suggest that the projection from V4 might be able to carry enough visual information to TE after TEO lesion to support high-resolution categorization of images.

Disclosures: K. Martinez Gomez: None. **B. Li:** None. **N. Miyazaki:** None. **M.A. Eldridge:** None. **W. Lerchner:** None. **B.J. Richmond:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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Topic: D.06. Vision

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Title: Remote activations induced by microstimulation in the macaque primary visual cortex

Authors: *L. MERKEN, P. JANSSEN;
KU Leuven, Leuven, Belgium

Abstract: Electrical microstimulation of neurons in the primary visual cortex can lead to the perception of phosphenes. Based on this concept, research groups are developing cortical visual prostheses to regain sight in blind patients. However, the spatial spread of the induced activations caused by microstimulation at the neuronal level remains unclear. We investigated the extent of neuronal activation using multi-unit activity (MUA) recordings with a high-frequency stimulation protocol used for visual prosthesis research (200 Hz, cathodic-first biphasic pulses for 200 ms). We applied electrical microstimulation in the primary visual cortex of a macaque monkey implanted with 100 comb-shaped flexible electrodes (with receptive field locations 0 to 4 deg from the fovea) using a CereStim R96 stimulator and a 128-channel Cerebus recording system. We varied stimulation intensity (low-intensity stimulation, LIS: 5 and 10 µA, moderate-intensity stimulation, MIS: 30 and 50 µA and high-intensity stimulation, HIS: 100 µA) and recorded during and after microstimulation at different locations (2.1 to 18 mm from the stimulated channel) during passive fixation of a target on a display. We found that higher stimulation intensities modulated significantly more remote (i.e. more than 1.4 mm away) channels (7% of channels with LIS, 12% with MIS and 22% with HIS, permutation test $p < 0.01$; $X^2 = 132$, $p < 0.001$). All stimulation intensities were able to influence (excite or inhibit) channels remotely at distances up to 18 mm from the stimulated electrode, but higher intensities exerted a significant effect at shorter distances from the stimulated channel (mean distance from the stimulated electrode with a significant effect 9.4, 9.5 and 5.7 mm for LIS, MIS and HIS, respectively, $p < 0.001$). However, these remote effects were mainly inhibitory for LIS and MIS (6% and 11%) and rarely excitatory (both 1%), whereas HIS induced more remote activations (20%) compared to inhibitions (2%, $X^2=454$, $p < 0.001$). These results highlight the importance of electrophysiological recordings during microstimulation to find the most optimal focal activations.

Disclosures: L. Merken: None. **P. Janssen:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

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Title: Investigating the effects of nicotinic receptor activation in granular V1 on supragranular and infragranular V1

Authors: *V. C. GALVIN[1], T. NGUYEN[2], A. A. DISNEY[2];
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Abstract: Primary visual cortex (V1) in primates receives cholinergic innervation from the basal forebrain. Expression of both cholinergic muscarinic and nicotinic receptors are found in V1, with distinct expression patterns across lamina. The β2-containing nicotinic receptor is highly expressed in thalamic axons in layer 4c, poised to strongly enhance incoming visual information. Indeed, it has been shown that activating these receptors with the agonist nicotine increases responsiveness and lowers the contrast threshold of neurons in layer 4c in anesthetized primates. In awake behaving primates, blockade of muscarinic or nicotinic receptors decreases neuronal contrast sensitivity in layer II/III neurons, primarily via response gain changes. How ACh actions at nicotinic receptors in thalamic input layers impact the propagation of visual information within V1 is unknown. In our study we investigate the existence and extent of this by conducting in vivo electrophysiology using laminar recording probes with drug delivery capacity within layer 4c. We measured the effects of nicotinic receptor activation on layer 4c neurons, and how changes in 4c neuronal activity impacted activity of presumed supragranular and infragranular neurons in the same visual column, as determined by receptive field mapping and orientation tuning. We report enhancement of supragranular neuronal activity when 4c neurons are enhanced by nicotine, and mixed effects on infragranular neuronal activity.

Disclosures: **V.C. Galvin:** None. **T. Nguyen:** None. **A.A. Disney:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 635.09

Topic: D.06. Vision

Title: Ocularity-contingent monocular and binocular responses and ocularity functional organizations of V1 superficial-layer neurons in macaques

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Abstract: V1 neurons in primates respond to binocular stimulation, but with various degrees of eye preferences. We used two-photon calcium imaging (GCaMP5) to study a) ocularity-contingent monocular and binocular responses of macaque V1 superficial neurons and their contributions to stable perception when the world is viewed with one or both eyes open; b) functional organization of ocularity and its relationships to orientation and SF maps.The stimulus was a high-contrast (0.9) drafting (2 cycles/sec) Gabor of 12 orientations (in 15° steps) and 6 SFs

(0.25-8 cpd), and was presented binocularly or monocularly to each eye. Data analysis identified a total of 7335 neurons that were orientation selective with at least one eye.Most V1 superficial-layer neurons responded to stimulations from both eyes, and only a few were strictly monocular. When monocularly stimulated, the best responses of more monocular neurons were markedly stronger than those of more binocular neurons, approximately in the form of a quadratic function. However, stronger responses of monocular neurons were suppressed, and weaker responses of binocular neurons were enhanced by binocular stimulation. As a result, the net population responses remain little changed, which may be related to the homeostasis of perception under monocular and binocular viewing. The monocular and binocular response changes can be described with a binocular integration and gain control modal, which also predicts binocular summation at low stimulus contrasts.V1 neurons clustered with similar ocularity index within the same recording plane. The median clustering index was 1.87, significantly higher than permuted data. Neurons also formed ocular dominance columns across depth planes. Neuron-wise, ocularity, SF, and orientation functional maps intersected each other in a wide range of angles, with the median around 40°. The vector sums of individual angles were near 0^o, but with large circular variances (>0.8), suggesting no reliable relationships among these functional maps.

Disclosures: **S. Zhang:** None. **S. Tang:** None. **C. Yu:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

Location: SDCC Halls B-H

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Topic: D.06. Vision

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Whitehall Research Foundation

Title: Delineating candidate cell types in monkey V1 using nonlinear dimensionality reduction and high density electrophysiology

Authors: *N. CARR[1], E. K. LEE[2], S. ZHU[5], R. XIA[6], X. CHEN[9,10], A. PERLISS[1], T. MOORE[5,6,7,11,8], C. CHANDRASEKARAN[3,2,1,4];
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Abstract: How diverse neural populations in the primary visual cortex (V1) participate in visual function in primates in vivo is still unknown. Current approaches that rely entirely on features such as waveform width are insufficient to capture cell type diversity. We recently developed a nonlinear dimensionality reduction approach (WaveMAP) that can better separate candidate cell classes. Here, we apply this approach to recordings from high density Neuropixels, which provide improved spatial resolution, to delineate candidate cell types and their response properties in V1. We used Neuropixels to record from the lateral opercular surface V1 of two anesthetized monkeys (5 sessions) viewing circular drifting Gabor gratings in 36 directions with 4 different spatial frequencies. After spike sorting and manual curation, we identified 801 negative spiking neurons and clustered them into candidate cell types on the basis of normalized waveform shape using WaveMAP. Laminar compartments were determined with current source densities and histology. We identified 5 candidate cell types with distinct laminar ($\chi 2(20) = 40.60$, $p<.001$) and firing rate properties (Kuskal-Wallis test, $p<.05$) which may correspond to V1 cell populations identified in previous studies. Of note, we found two candidate cell types localized to layer 4C with broad but distinct waveform shapes. One cell class had the highest and most sustained firing rates, while the other cell class had lower firing rates and slightly slower response latency. These may correspond to cell populations receiving magnocellular and parvocellular inputs respectively. We also found a candidate cell type concentrated in Layer 4A/B with a narrow waveform shape and a strong initial response to the visual stimulus which decayed over time. This cell population may correspond either to spiny stellate cells in the magnocellular pathway or parvalbumin expressing inhibitory cells identified in anatomical studies. Another candidate cell type was found in Layer 5/6 and had the broadest waveforms and robust firing rates. Surprisingly, these neurons had the shortest response latency, which resembles Layer 6 neurons that receive direct geniculocortical input. Finally, we found a candidate cell type distributed across all layers except Layers 2/3, with narrow, triphasic waveforms. This cell class had the lowest firing rate response and the slowest latency suggesting these are perhaps outputs from V1. Together our results show that combining high density electrophysiology with machine learning can help delineate cell types within a cortical column, furthering our understanding of circuits underlying feedforward processing in V1.

Disclosures: **N. Carr:** None. **E.K. Lee:** None. **S. Zhu:** None. **R. Xia:** None. **X. Chen:** None. **A. Perliss:** None. **T. Moore:** None. **C. Chandrasekaran:** None.

Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

Location: SDCC Halls B-H

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Program #/Poster #: 635.11

Topic: D.06. Vision

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Title: Cytoarchitectonic definition of the human homolog of macaque area V6

Authors: *M. GAMBERINI[1], N. PALOMERO-GALLAGHER[2,3,4], M. TESTA[1], S. BLUDAU[2], K. AMUNTS[2,4,5], P. FATTORI[1], C. GALLETTI[1];
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Abstract: Different experimental approaches are used to define brain cortical areas. Extrastriate area V6 has been described in humans on the basis of functional imaging studies (Pitzalis et al., J. Neurosci. 2006). As macaque area V6, human area V6 (hV6) is a pure visual area, which is very sensitive to visual motion and retinotopically organized. It represents, as in macaque, almost the whole visual field up to the far periphery. Aim of this study was to describe the location and extent of hV6 on the basis of its cytoarchitectural structure, and to make 3D view digital reconstructions of the entire brain and of the hV6 to allow a comparison between anatomical and functional definitions of this area. We examined 11 human post-mortem brains (21 hemispheres; 6 males; mean age 58.5 ± 12.2 years; 5 females; mean age 68.4 ± 13.2 years) of subjects without a history of neurological or psychiatric disorders, obtained via body donors in accordance with guidelines of the Ethics Committee of the University of Düsseldorf. Brains were removed from the skull, fixed in formalin, scanned with a T1-weighted magnetic resonance sequence before histological processing, then embedded in paraffin and serially sectioned (section thickness 20 μm) in the coronal or horizontal planes with a large-scale microtome. Every 60th section stained for cell bodies was mapped, and the borders of hV6 with neighboring regions was identified and statistically validated. As in macaque, hV6 displays architectonic features typical of the occipital cytoarchitectonic domain such as a thick, densely packed and homogeneous layer IV, a light layer V, and a clearly defined layer VI/white matter border. hV6 shows thin vertical cell columns resembling raindrops. The degree of architectural similarity between hV6 and nearby areas within the intraparietal sulcus and POS was quantified using Matlab by performing hierarchical cluster and multidimensional scaling analyses. Present results showed three main clusters among the areas of this brain region, and hV6 appeared as a solitary area, standing out from all other ones. Areal delineations were used for computation of volumes of hV6 in each individual brain, which were then superimposed in anatomical MNI space to generate a continuous probabilistic map. The extent and location of hV6 was comparable in the different individual cases. It occupied the dorsalmost third of the posterior wall of parieto-occipital sulcus (POS) and was found between previously defined areas hPO1 and hOc3d. The data are congruent with those obtained with neuroimaging techniques, suggesting that hV6 is a distinct area not only functionally, but also under the cytoarchitectonic point of view.

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Poster

635. Functional Architecture and Circuits in the Visual Cortex of Non-Human Primates II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 635.12

Topic: D.06. Vision

Support: B.P. has received funding from the EU Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 841578

Title: A superset family of neural network architectures for the systematic analysis of receptive field size and computational-path-length distribution in the primate visual hierarchy

Authors: *B. PETERS[1], L. STOFFL[3], N. KRIEGESKORTE[2];
[2]Zuckerman Mind Brain Behavior Inst., [1]Columbia Univ., New York, NY; [3]Brain Mind Inst., Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Abstract: Deep feedforward convolutional neural network models (FCNNs) explain aspects of the representational transformations in the primate visual hierarchy. However, particular models implement idiosyncratic combinations of architectural hyperparameters, which limits theoretical progress. In particular, the size of receptive fields (RFs) and the distribution of computational path lengths (CPL; the number of nonlinearities encountered) leading up to a representational stage are confounded across layers of the same architecture (deeper layers have larger RFs) and depend on idiosyncratic choices (kernel sizes, depth, skipping connections) across architectures. Here we introduce HBox, a superset family of architectures designed to break the confoundation of RF size and CPL. Like conventional FCNNs, an HBox model contains a feedforward hierarchy of convolutional feature maps. Unlike FCNNs, each map has a predefined RF size that can result from shorter or longer computational paths or any combination thereof (through skipping connections). We implemented a large sample of HBox models (>400) inducing representational stages with a diverse distribution of RF sizes and CPL. Using representational similarity analysis we quantify the distribution of RF sizes and CPL in regions of interest in a large-scale human fMRI benchmark (natural scenes dataset; Allen et al., 2021). In particular, we observe that HBox representations reveal an increase in receptive field size and computational path length along lower and higher-level regions in the ventral visual stream. The HBox architecture family illustrates how high-parametric task-performing vision models can be used systematically to gain theoretical insights into the neural mechanisms of vision.

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Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 636.01

Topic: D.08. Multisensory Integration

Support: IBS Grant IBS-R015-D1
KBRI Grant 22-BR-01-01 and 22-BR-03-07

Title: Adult deafness induces rapid cross-modal plasticity in the mouse somatosensory and visual pathways

Authors: *H.-J. SHIM[1], W. JUNG[1], G. KIM[2], S.-G. KIM[1,3];
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Abstract: Early sensory loss can induce cross-modal reorganization of sensory cortices, which may underlie the enhancement of spared senses. Recent evidence indicates deafening in adulthood can also induce a significant degree of cortical plasticity. However, the extent of reorganizations across the brain regions and the neural mechanisms that drive this adult plasticity remain unclear. Here, we investigated the effects of deafening in adult mice, using 15.2T blood-oxygenation-level-dependent (BOLD) functional MRI (fMRI), which allows brain-wide mapping of sensory functions before and after deafening in each animal. *Pou4f3*$^{DTR/+}$ mice, in which cochlear hair cells express diphtheria toxin receptors, were deafened by an injection of diphtheria toxin (i.m., 25 ng/g at the age of ~7 weeks) and deafness was confirmed by the absence of acoustic startle response ~5 days after injection. BOLD responses to forepaw stimulation or visual stimuli were obtained in 15.2T scanner before and 7 days after the toxin injection. To test whether MR scanner noise suppresses sensory responses in hearing mice, optical intrinsic signal (OIS) imaging was performed while playing back scanner noise recordings (~115 dB). Effects of acute inactivation of auditory cortex were examined by measuring BOLD responses using VGAT-ChR2-EYFP mice. After deafening, fMRI responses to forepaw stimulation were enhanced in the primary somatosensory cortex (S1FL; 0.74±0.14% to 1.45±0.14%), the somatosensory thalamus (VPL; 0.27±0.08% to 0.61±0.08%), and the secondary somatosensory cortex (S2; 0.23±0.07% to 0.69±0.08%) (n = 10 mice). fMRI responses to visual stimuli were enhanced in the primary visual cortex (V1; 0.63±0.09% to 0.92±0.08%) and the visual thalamus (LGN; 0.36±0.07% to 0.56±0.07%) (n = 10 mice). No enhancement was observed in WT mice who received toxin injection (n = 7 mice), and our control experiments indicate MR scanner noise or acute inactivation cannot account for the enhancement (n = 5 and 6 mice, respectively). Our results demonstrate that deafening in adult mice enhances cortical and subcortical sensory evoked BOLD responses in the spared sensory pathways, and this enhancement occurs within a week after deafening. Our results suggest that sensory loss in adulthood triggers rapid compensatory cross-modal plasticity in the brain regions beyond primary sensory cortices.

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Poster

636. Cross-Modal Processing II

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Program #/Poster #: 636.02

Topic: D.08. Multisensory Integration

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Title: Functional characterization of macaque insula using resting-state and task-based fMRI.

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[1]KU Leuven, Leuven, Belgium; [2]Leuven Brain Inst., Leuven, Belgium; [3]Harvard Med. Sch., Boston, MA; [4]CNRS CERCO UMR 5549, Toulouse, France

Abstract: Background: Neurophysiological investigations over the past decades have demonstrated the involvement of the primate insula in a wide array of sensory, cognitive, affective and regulatory functions (Evrard, 2019). Invasive studies in the macaque monkey suggest the existence of several functionally distinct insular subregions, including a posterior vestibular field (Chen et al., 2010), a mid-dorsal sensorimotor field (Jezzini et al., 2012), a mid-ventral affiliative/social field (Caruana et al., 2011; Jezzini et al., 2015) and an anterior field related to ingestive behavior, taste coding (Smith-Swintosky et al., 1991) and disgust (Caruana et al., 2011; Jezzini et al., 2012). Methods: Here we examined to what extent resting-state fMRI hierarchical clustering (awake macaques, n = 8), which has come forward as a promising technique for investigating the functional organization of cortex non-invasively (Hutchison and Everling, 2014), could retrieve aforementioned functionally distinct insular subregions. The correspondence of the partitioning schemes derived from the hierarchical clustering analysis with functional specializations was further examined using a series of task-related fMRI experiments (galvanic vestibular stimulation, somato-motor grasping task, taste perception, disgust experience, observation of social and affiliative visual stimuli including conspecific face gestures and grooming behavior), as well as seed-based resting-state fMRI analyses. Results: Permutation/fingerprint methods based upon extrinsic interareal functional connectivity (Schaeffer et al., 2020) suggested the presence of four insular clusters, including a posterior, mid-dorsal, mid-ventral and anterior cluster. Functional specialization of these insular clusters was further corroborated by seed-based whole brain resting-state analyses, showing clearly distinct functional connectivity gradients across the anterio-posterior extent of the dorsal insula as well as between dorsal and ventral insula. Task-based fMRI mapping suggested these clusters showed a functional specialization related to processing of vestibular information (posterior cluster), somato-motor responses (mid-dorsal cluster), social information (mid-dorsal and mid-ventral clusters) and taste/ingestive/disgust responses (anterior cluster). Conclusion: Overall, our data suggests that hierarchical clustering analysis of resting-state fMRI data can retrieve, at least a coarse functional organization of the macaque insula, in line with functional specializations as suggested by task-based fMRI or previous electrophysiological investigations.

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Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 636.03

Topic: D.08. Multisensory Integration

Title: X-ray perception: animal studies of sensory and behavioral responses to x-rays

Authors: *C. S. ROWE;
Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Since their discovery in 1895, many studies have been conducted to understand the effect of X-rays on neural function and behavior in animals. These studies examined a range of acute and chronic effects and a subset of studies has attempted to determine if X-rays can produce any sensory responses. Here we review literature on animal behavioral responses to X-rays from 1895 until 2021 to assess the evidence for detection of X-rays by sensory receptors in animals. We focus on the changes in appetitive and consummatory behavior, radiotaxis, behavioral arousal, and olfactory responses to X-rays that have been reported in the literature. Taken together, the reviewed literature provides a large body of evidence that X-rays can induce sensory responses in a wide variety of animals and also suggests that these responses are mediated by known sensory receptors. Furthermore, we postulate the role of reactive oxygen species, the most biologically active byproduct of X-rays, as a key mediator of sensory receptor responses to X-rays.

Disclosures: C.S. Rowe: None.

Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

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Program #/Poster #: 636.04

Topic: D.08. Multisensory Integration

Support: ONR N00014-22-1-2208

Title: Multiple nerve cords connect octopus arms, providing parallel paths for interarm signaling

Authors: *A. KUUSPALU, S. CODY, M. E. HALE;
Univ. of Chicago, Chicago, IL

Abstract: The nervous system of octopuses provides an alternative organization to other model taxa for sensorimotor integration and control of limb movement. Limbed species typically have a central nerve cord that lies along the anteroposterior axis of the body, with sensory and motor fibers extending from the cord into each limb. In contrast, octopuses possess five nerve cords in each of their arms. We examined the neural pathways of all five nerve cords to better understand their potential neural connectivity outside of the arm proper. Using immunohistochemical markers, cryosectioning and confocal imaging in young *Octopus bimaculoides* we tracked the nerve cords at the base of the arm. The central axial nerve cord (ANC) of the arm is known to connect to the brain by the brachial nerves and among the arms through a nerve ring at the arms' base. We add to the previous descriptions of the ANC, detailing the transition of the ANC to the brachial nerve and adding detail on the interbrachial commissures. In addition to the ANC, every arm has four smaller intramuscular nerve cords (INCs) that run parallel to the ANC. The two oral INCs, ventrolateral to the ANC, extend proximally from the arm and join oral INCs from other arms to form interarm pathways that are distinct from the main ANC-connected nerve ring. This provides an additional potential means by which arms may connect to one another outside of the brain. The two aboral INCs, INCs that lie dorsolateral to the ANC in the arm, stay close to the surface of the body between the muscle layers of the interbrachial membrane. The aboral INCs extend laterally while paralleling the ANC on a path toward the brain. The aboral INC on one side of the arm ultimately converges with the neighboring aboral INC of the adjacent arm. These aboral ANC data suggest a means for transmitting information signals from/to the interbrachial membrane and suggests a potential connection between adjacent arms. These investigations identified additional features of the octopus' nervous system at the base of the arm and show increased complexity in the neural pathways associated with interarm neural signaling.

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Poster

636. Cross-Modal Processing II

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Program #/Poster #: 636.05

Topic: D.08. Multisensory Integration

Title: X-ray perception: animal studies of sensory and behavioral responses to x-rays

Authors: *V. MANTRARATNAM[1], C. S. ROWE[2];
[2]Radiology, [1]UAB, Birmingham, AL

Abstract: Since their discovery in 1895, many studies have been conducted to understand the effect of X-rays on neural function and behavior in animals. These studies examined a range of acute and chronic effects and a subset of studies has attempted to determine if X-rays can produce any sensory responses. Here we review literature on animal behavioral responses to X-rays from 1895 until 2021 to assess the evidence for detection of X-rays by sensory receptors in animals. We focus on the changes in appetitive and consummatory behavior, radiotaxis,

behavioral arousal, and olfactory responses to X-rays that have been reported in the literature. Taken together, the reviewed literature provides a large body of evidence that X-rays can induce sensory responses in a wide variety of animals and also suggests that these responses are mediated by known sensory receptors. Furthermore, we postulate the role of reactive oxygen species, the most biologically active byproduct of X-rays, as a key mediator of sensory receptor responses to X-rays.

Disclosures: V. Mantraratnam: None. **C.S. Rowe:** None.

Poster

636. Cross-Modal Processing II

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Program #/Poster #: 636.06

Topic: D.08. Multisensory Integration

Support: NSF Grant 2113028
NSF Grant 2015317

Title: Direct Assembly and Tuning of Dynamical Neural Network Models that Calculate Leg Kinematics

Authors: C. K. GUIE, *N. S. SZCZECINSKI;
Dept. of Mechanical and Aerospace Engin., West Virginia Univ., Morgantown, WV

Abstract: It is unknown precisely how the nervous system of invertebrates combines multiple sensory inputs to calculate more abstract quantities, e.g., combining the angle of multiple leg joints to calculate the position of the foot relative to the body. In this study, we propose that known non-spiking interneurons (NSIs) in the insect nervous system could calculate such quantities and construct a neuromechanical model to support the claim. Range fractionated sensory inputs are modeled as multiple integrate-and-fire neurons. The NSI is modeled as a multi-compartment dendritic tree and one large somatic compartment. Each dendritic compartment receives synaptic input from one sensory neuron from the knee and one from the hip. The voltage of the somatic compartment accurately follows the true position of the foot. We also discuss motivation for future research, which includes modeling other hypothetical networks in the insect nervous system and integrating this model into task-level robot control.

Disclosures: C.K. Guie: None. **N.S. Szczecinski:** None.

Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

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Program #/Poster #: 636.07

Topic: D.08. Multisensory Integration

Support: NIH AA13023

Title: Development of multisensory integration in the cortex and effects of developmental alcohol exposure

Authors: *A. E. MEDINA[1], D. KEUM[1], W. A. FOXWORTHY[2], K. PULTORAK[1], A. TARASIEWICZ[3], M. A. MEREDITH[4];
[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Biol., Eastern Shore Community Col., Melfa, VA; [3]Sch. Of Med. Univ. Of Maryland, Baltimore, MD; [4]Virginia Commonwealth Univ., Richmond, VA

Abstract: Fetal alcohol spectrum disorders (FASD) is one of the most common causes of mental disabilities in the world with a prevalence of 1-6% of all births. Sensory processing problems is a major feature in this condition. Because developmental alcohol exposure can impair neuronal plasticity; and neuronal plasticity is crucial for the establishment of neuronal circuits in sensory areas; we predicted that exposure to alcohol during the third trimester equivalent of human gestation would disrupt the development of multisensory integration (MSI). First, we conducted experiments to characterize the development of MSI in the ferret rostral posterior parietal cortex (PPr). Next, we evaluated the effects of early alcohol exposure on MSI. In the first set of experiments PPr neurons were examined by *in vivo* electrophysiology in 18 ferrets in four age groups (Infancy, early, mid and late adolescence). A total of 538 PPr neurons from anesthetized ferrets were recorded after visual, tactile and combined visual-tactile stimulation. A Multisensory (MS) enhancement or suppression is characterized by an increased or decreased number of elicited spikes after combined visual-tactile stimulation compared to the strongest unimodal (visual or tactile) response. Our findings show that during development neurons in PPr goes from a state where MS suppression is dominant (during infancy) to a state where MS enhancement is dominant (during late adolescence). KS tests done on cumulative probability curves of % changes in firing showed significant differences between all age groups tested ($p<0.001$ for all comparisons). In a second set experiments we recorded 1157 neurons from 17 ferrets from four groups (Saline/Alcohol; Infancy/Adolescence). While alcohol animals showed similar developmental changes between infancy to adolescence they always “lagged behind” controls showing more MS suppression and less enhancement. KS tests done on cumulative probability curves of % changes in firing showed significant differences between all exposure/age groups tested ($p<0.001$ for all comparisons). Therefore, our findings suggest that alcohol exposure during the last months of human gestation would stunt the development of multisensory integration in FASD, which could underlie some of the sensory problems seen in this condition.

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Poster

636. Cross-Modal Processing II

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Program #/Poster #: 636.08

Topic: D.08. Multisensory Integration

Support: CIHR PG-150030
FRQS NeuroNex-295825

Title: Olfactory integration in nociceptive circuits modulates escape behavior in Drosophila larvae

Authors: *J. NING, T. OHYAMA;
Dept. of Biol., McGill Univ., Montreal, QC, Canada

Abstract: It is generally believed that the decisions and behaviors executed by organisms are improved with the number of informative sensory cues, especially from different modalities. However, identifying the mechanisms by which such multimodal processing of information is achieved remains a challenging task, since the flow of information from divergent sensory inputs must be traced from the periphery to the hubs within the nervous system at which they are integrated. Here, we used a tractable and naturalistic escape behavior sequence in *Drosophila* larvae—rolling followed by fast crawling in the event of an attack by a parasitic wasp—to show how combining sensory cues from two sensory modalities—pain and smell—potentially optimizes the proper execution of the escape sequence and hence increases the chance of survival. Specifically, we demonstrate that the transition between rolling, which is triggered by a nociceptive stimulus (i.e., penetration by the ovipositor of a parasitic wasp in the natural setting, or optogenetic stimulation of key nociceptive neurons in the lab), and fast crawling, which obligatorily occurs after rolling, is facilitated by the presence of an odor. These findings present an opportunity to understand how distinct sensory modalities are integrated in the nervous system and contribute to the efficient production of natural behaviors.

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Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 636.09

Topic: D.08. Multisensory Integration

Support: Deutsche Forschungsgemeinschaft (DFG) 395940726 - SFB 1372 (project Neu04)

Title: The organization of motor-related projections of the caudolateral nidopallium in pigeons (Columba livia)

Authors: *A. STEINEMER, A. SIMON, O. GÜNTÜRKÜN, N. ROOK;
Biopsychology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: The multimodal nidopallium caudolaterale (NCL) in pigeons is considered to be analogous to the mammalian prefrontal cortex and sends descending motor projections to the intermediate arcopallium (AI) and medial striatum (MSt). To gain detailed insights into the organization of these projections, we conducted retrograde and anterograde tracing experiments. First, we tested whether NCL neurons that project to AI (NCL_{arco} neurons) and MSt (NCL_{MSt} neurons) are constituted by a single neuronal population with bifurcating neurons, or whether they form two distinct populations. We did not find a single bifurcating cell, but instead a remarkably differentiated projection pattern to both target areas. Second, we wanted to describe the two projections in greater detail by examining a potential topographic organization. Indeed, we revealed a weak topographic projection towards the medial and lateral striatum and a strong topographic projection towards AI with clearly distinguishable termination fields. Third, we searched for morphological differences between NCL_{arco} and NCL_{MSt} neurons by measuring the cell body sizes of the two populations. We found that NCL_{MSt} neurons have significantly larger somata. Fourth, we characterized the neurochemical profile of NCL_{arco} neurons by fluorescence stainings against GABA, CaMKII, and the calcium-binding protein calbindin (CB). We found that these neurons expressed virtually no CB or GABA, but almost one third was positive for CaMKII. In addition, we counted a considerable number of potential NCL interneurons that were double labeled for CB and GABA. In summary, the present study demonstrates that the intratelencephalic motor connections in the pigeon brain are highly organized and that they share multiple characterictics with HVC-pathways in the oscine brain. We concluded that NCL projection neurons do not seem to constitute an ancient neuronal pattern from which the more specialized oscine song system has emerged. Instead, NCL projection neurons appear to be already specialized in a fashion that serves particular functions. Eventually, we discuss evidence gained from these experiments by referring to the evolution of the avian motor system from which, possibly, the song system has emerged.

Disclosures: A. Steinemer: None. **A. Simon:** None. **O. Güntürkün:** None. **N. Rook:** None.

Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 636.10

Topic: D.08. Multisensory Integration

Title: Loose balance supports distributed high-dimensional predictive coding in recurrent neural networks

Authors: *B. WANG[1], J. ALJADEFF[2];
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Abstract: Predictive responses to sensory stimuli are prevalent across cortical networks, and are hypothesized to be important for sensory and motor learning [1]. Traditionally, predictive processing is investigated in a scenario where a single association between a pair of stimuli is learned (e.g., visual-auditory [2]). Based on these experimental observations with a single pair of stimuli, it has been suggested that cortical neurons are divided into separate classes: one specialized for “faithful” representation of external sensory stimuli, and another for conveying prediction-error signals [1]. The latter type is thought to “compare” an internally generated prediction to the actual sensory input.However, in natural settings, stimuli are typically high-dimensional and multimodal. This necessitates learning of many associations between stimulus pairs, to facilitate effective and robust predictions. Currently, we have a limited understanding of how such high-dimensional predictive representations are formed and maintained in neural systems. Moreover, there is no evidence supporting the notion that prediction-error signals are segregated from stimulus representations.Here we propose an analytically tractable model of a recurrent neural network that performs high-dimensional predictive processing of many learned stimulus-pairs. We show that there is an optimal degree of balance - in the loose regime [3] - that allows the network to bind paired stimuli and generate prediction-error responses, without an explicit supervisor signal.The main consequence of the high-dimensional predictive representation in our model is that, contrary to the previous hypothesis, functional responses of neurons in the network are heterogeneous across stimuli: each neuron may form faithful representations for some stimuli while signaling prediction errors for other stimuli. Our model recapitulates recent experimental results: (i) that neuronal responses are positively correlated in the matched and mismatched scenarios; and (ii) that the learning process decorrelates the population responses between the two scenarios [2].In conclusion, we propose a simple mathematical model that offers a new perspective on learning high-dimensional predictive representations. Our model suggests that predictive and stimulus representations are mixed, which can be tested experimentally; and that such representations can be learned “automatically,” without a supervisor signal.References:[1] Keller, Mrsic-Flogel. (2018) Neuron[2] Garner, Keller. (2022) Nat. Neuro.[3] Ahmadian, Miller. (2021) Neuron

Disclosures: B. Wang: None. **J. Aljadeff:** None.

Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 636.11

Topic: D.08. Multisensory Integration

Title: Neural computational models underlying coordinate transforms of motion representations in touch

Authors: *H. AHUJA[1], G. C. DEANGELIS[2], M. GOMEZ-RAMIREZ[1];
[1]Brain and Cognitive Sci., [2]Brain Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Our ability to perceive motion information on the skin is key to manipulating dynamic objects in the environment. Neural mechanisms that generate perception of tactile motion play a key role in haptics by providing sensory feedback signals used to make grasp adjustments (e.g., signaling that an object is slipping). Previous studies show that the brain derives tactile motion representations by integrating object cues that impinge on the skin (e.g., speed, force, direction), a mechanism known as the Full Vector Average model. This model was derived from studies that placed the hand in the same posture, and in one specific reference frame. However, we showed that perception of tactile motion on the hand is modulated by the proprioceptive state of the arm in a reference frame-specific manner. Specifically, motion discrimination on the hand is modulated by arm position when judgements are made relative to the center of the body (i.e., sternum), but not relative to a finger (i.e., thumb). These data indicate that current models of tactile motion perception require major revisions. Accordingly, we developed a Bayesian Generative model using Euler matrix computations that account for human tactile motion discrimination functions in different reference frames, and with the hand placed in different proprioceptive states. Here, we developed several computational frameworks, at the neuronal level, that could underlie these Euler matrix computations. We model the effect of proprioceptive state and reference frame instruction on tactile motion by using a combination of gain-field, additive and phase shifts parameters of tuning functions of neural populations in primary somatosensory (SI) cortex. Then, we study the effects of these different combinations on the performance of a decoder. We also adapt a basis-function network that can simultaneously represent tactile motion in different reference frames, as well perform non-linear transformations between them. These neural simulations robustly account for human motion discrimination functions. Further, we use this network to make testable predictions about neural tuning functions of neocortical populations in SI, which we will validate through neural recordings done in monkeys performing tactile motion discrimination tasks in different reference frames, and with their hands placed in different postures.

Disclosures: H. Ahuja: None. **G.C. DeAngelis:** None. **M. Gomez-Ramirez:** None.

Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 636.12

Topic: D.08. Multisensory Integration

Support: Brain & Behavior Research Foundation

Title: Cortical circuit mechanisms of atypical sensory processing and hypersensitivity in a mouse model of Fragile X syndrome

Authors: *N. RAHMATULLAH[1], J. ROBLEDO[2], S. POST[2], G. CHAUDHARI[3], C. PORTERA-CAILLIAU[3,4], A. GOEL[2,1];
[1]Neurosci. Grad. Program, [2]Dept. of Psychology, Univ. of California, Riverside, Riverside, CA;

[3]Dept. of Neurol., UCLA, Los Angeles, CA; [4]Dept. of Neurobio., David Geffren Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Fragile X syndrome (FXS) and autism spectrum disorder (ASD) are associated with atypical sensory processing, which often manifests as sensory hyperarousal and hypersensitivity. This may be linked to higher-order cognitive impairments in attention, learning, memory, emotion, social interaction, and language. Despite the prevalence of sensory issues, the circuit mechanisms underlying atypical sensory processing and hypersensitivity to sensory stimuli are largely undefined. We investigated how these differences in sensory processing influence goal-directed behaviors by using the best-studied animal model of FXS - *Fmr1* knockout (*Fmr1* KO) mice. We previously found that compared to wild-type (WT) mice, *Fmr1* KO mice were delayed in learning a go/no-go visual discrimination task. Our recent unpublished work demonstrates that *Fmr1* KO mice exhibit a greater susceptibility to distracting auditory and visual stimuli, indicating hypersensitivity and inability to tune out distractors. In addition, vasoactive intestinal peptide inhibitory neurons in *Fmr1* KO mice exhibited elevated basal activity primary visual cortex (V1) and were less modulated by visual stimuli and distractor presentation. Top-down modulation from frontal regions of the brain is important for sensory guided behaviors and drive selective attention and necessary inhibitory processes (Miller and Cohen, 2001). Prior studies have demonstrated that long-range inputs from one frontal cortical region - anterior cingulate cortex (ACC) - are involved in increasing cortical response to behaviorally relevant information and attenuating responses to extraneous inputs (Zhang et al, 2014). In mice, projections from ACC heavily innervate V1 and influence local visual processing (Zhang et al, 2016). We hypothesize that a dysfunction in afferent inputs from ACC to V1, specifically a reduction, may contribute to sensory hypersensitivity and inability to tune out distracting stimuli in *Fmr1* KO mice. Our preliminary in vivo two-photon calcium imaging of ACC axon terminals in V1 during passive viewing of visual stimuli suggests hypoactive ACC to V1 inputs in *Fmr1* KO mice. Identifying disruptions in these long-range inputs to V1 is necessary for a mechanistic understanding of sensory hypersensitivity in a range of neurodevelopmental disorders.

Disclosures: **N. Rahmatullah:** None. **J. Robledo:** None. **S. Post:** None. **G. Chaudhari:** None. **C. Portera-Cailliau:** None. **A. Goel:** None.

Poster

636. Cross-Modal Processing II

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Program #/Poster #: 636.13

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Support: T32 NS007433-22
DP2MH122404-01
BRAIN R01
Searle Scholars Program

Pew Biomedical Scholars Program
Klingstein-Simons Fellowship

Title: Auditory-driven activity in cell-type specific populations of mouse posterior parietal cortex

Authors: *C. BASSI[1,2], C. A. RUNYAN[1,2];
[1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Responses to incoming sensory inputs in primary sensory areas and in the posterior parietal cortex (PPC) are modulated by arousal and task engagement, yet the circuit-level basis of this response flexibility is unknown. Inhibitory interneurons are critical components of local microcircuits and are well-suited to mediate this state-dependent response flexibility due to their large diversity in connectivity patterns and neuromodulator receptor expression. Two of the largest groups of inhibitory neurons present in the cortex express the molecular markers parvalbumin (PV) or somatostatin (SOM). PV and SOM neurons in primary sensory cortices participate in distinct connectivity "motifs" within local circuits: PV neurons preferentially provide feedforward inhibition, while SOM neurons pool local excitation, providing surround suppression. Furthermore, SOM neurons inhibit PV cells, releasing some excitatory neurons from PV-mediated inhibition, modulating response gain and population activity dynamics. Because of these distinct connectivity patterns, PV and SOM neurons are well situated to modulate the efficacy of incoming sensory signals in driving network activity.Here we characterize responses of PV, SOM, and putative excitatory neurons imaged simultaneously in mouse PPC, to determine their responses to feedforward (FF) auditory inputs. We used two-photon calcium imaging to measure spike-related fluorescence changes in GCaMP6f of genetically identified PV, SOM, and putative excitatory neurons in PPC in layers 2/3, and ChrimsonR to stimulate incoming FF auditory axons, while mice freely ran on a spherical treadmill. During each imaging session, auditory axons were photostimulated with brief pulses of amber light. Running velocity and pupil diameter were also tracked, as activity in PPC is strongly dependent on behavioral state. To quantify the effects of auditory axon photostimulation on individual neurons, we computed a modulation index. Preliminary results show diverse light-evoked responses across PPC neurons, including both negatively and positively modulated activity on average. When we examined the effects of auditory axon stimulation in single trials, we observed highly variable responses, despite the stereotyped stimulation conditions across trials. To determine the potential source of this variability, we examined the relationship between single trial responses to axon photostimulation and the activity state of PPC as well as to the behavioral state of the animal. These results highlight the dynamic processing of distinct populations in PPC to incoming FF sensory signals across behavioral states.

Disclosures: C. Bassi: None. **C.A. Runyan:** None.

Poster

636. Cross-Modal Processing II

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Pew Biomedical Scholars Program
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Title: Norepinephrine-driven changes in population activity in mouse posterior parietal cortex

Authors: *N. G. FALA[1], A. N. CHANDRASEKARAN[1,3], A. P. BATISTA[2,3], M. A. SMITH[4,3,5], C. A. RUNYAN[1,3];
[1]Dept. of Neurosci., [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [3]Ctr. for Neural Basis of Cognition, Pittsburgh, PA; [4]Biomed. Engin. and Neurosci. Inst., [5]Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: An organism's behavioral state, such as its level of arousal and attention, profoundly affects neuronal population activity across the cerebral cortex, and as a result, impacts perception and decisions, though the effects of state changes on neural populations differ along the cortical hierarchy. One key player in promoting such state shifts is norepinephrine (NE), which has been implicated in changes in arousal level, sustained attention in learned tasks, and signaling of novelty/saliency. The major source of NE in the brain, the locus coeruleus (LC), diffusely projects throughout the cortex. Until recently, these projections were considered to be nonspecific and uniform across cortex, but new studies suggest that LC may be more heterogenous, with subsets of neurons preferentially targeting distinct cortical regions. Furthermore, region-specific differences in receptor subtype expression and local microcircuitry could also lead to differences in NE-mediated shifts in local processing. Here, we measured the impacts of NE release on neural activity in the posterior parietal cortex (PPC), an association area that integrates inputs from multiple sensory modalities to generate behaviors. To manipulate PPC-projecting NE+ LC axons, we used DBH-cre mice injected with a cre-dependent channelrhodopsin viral construct in PPC (retroAAV-DIO-ChR2-mCherry). A green calcium indicator was also virally expressed in the PPC neuron population (AAV-syn-GCaMP6f). During each imaging session, we focally photostimulated LC axons with a blue LED, while simultaneously recording local population activity in PPC using two-photon calcium imaging of GCaMP6f. Mice ran freely on a spherical treadmill, and behavioral outputs including pupil size, running speed, and facial movements, which are known to correlate to the animal's arousal state, were simultaneously monitored. Photostimulation patterns mimicked the phasic and tonic firing patterns known to occur in LC neurons, with phasic or tonic stimulation occurring in different imaging sessions, and alternating with control stimulation trials, where the intensity of the LED was set to 0. With phasic stimulation, we observed a sparse population of PPC neurons that reliably increased their activity. However, when comparing the population activity patterns induced by LC stimulation to the population activity changes accompanying natural shifts in arousal, we observed that the two share similar features. Together, these experiments allow us to investigate the contribution of NE to state-dependent shifts in local population activity, and in future work, to compare the effects of NE on activity across cortical regions.

Disclosures: **N.G. Fala:** None. **A.N. Chandrasekaran:** None. **A.P. Batista:** None. **M.A. Smith:** None. **C.A. Runyan:** None.

Poster

636. Cross-Modal Processing II

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Topic: D.08. Multisensory Integration

Support: Pew Biomedical Scholars Program
Searle Scholars Program
Klingenstein-Simons Fellowship Award in Neuroscience
NIH grant NIMH DP2MH122404
BRAIN RO1

Title: A dual labeling method to image functional interactions between specific inhibitory populations

Authors: *C. T. POTTER[1,2], C. D. BASSI[1,2], C. A. RUNYAN[1,2];
[1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA

Abstract: Cortical interneurons (INs) can be divided into several subtypes based on their gene expression profiles, and work in concert to regulate pyramidal neuron activity, giving cortical circuits computational flexibility in the face of a changing environment and internal state. Combined with transgenic expression of anatomical fluorophores, two-photon calcium imaging can be used to identify specific cell types *in vivo*, and to functionally characterize individual components of local cortical circuits. Most commonly, a red fluorophore is expressed to identify one cell type, while a green calcium indicator, such as GCaMP, is used for imaging activity. Since INs can inhibit one another in addition to pyramidal neurons, they participate in a complex dynamical system that is better understood by measuring how their interactions unfold over time. To document how interneurons cooperate in this system, we developed a method to image activity in the full neuronal population while identifying two specific cell classes. We virally expressed GCaMP6f pan-neuronally along with two red fluorophores, tdTomato and mCherry, allowing simultaneous functional imaging of two inhibitory cell classes along with the activity of the full neural population. To discriminate the two red fluorophores, we took advantage their different excitation spectra, which were reliably separable in vivo by comparing relative emission across different excitation wavelengths. During each calcium imaging session, mice were allowed to run freely on a spherical treadmill, and we tracked running velocity as a proxy for arousal state. Using the data from the simultaneously recorded cell types in two cohorts of animals that labeled two subtypes each, we captured the temporal dynamics related to locomotion in posterior parietal cortex (PPC) for somatostatin (SOM), parvalbumin (PV), vasointestinal polypeptide (VIP), and the remaining putative pyramidal neurons. By computing

pairwise noise correlations among these subtypes, we characterized the spatial scale of their functional connectivity. We used pairwise cross correlations at different time lags to explore the temporal dynamics of inhibitory and disinhibitory interactions within and between cell types. Our approach will be useful to study the interactions among neuronal subtypes, their relationship to population activity dynamics and behavior, and how they vary from trial to trial.

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Poster

636. Cross-Modal Processing II

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Topic: D.08. Multisensory Integration

Support: NIH Grant R01 EY031532
Tab Williams Family Foundation

Title: The Development of Multisensory Integration is Site-specific

Authors: *S. A. SMYRE, N. L. BEAN, B. E. STEIN, B. A. ROWLAND;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: The ability to use cues from different senses in concert is one of the brain's fundamental features and is a powerful asset that enhances its ability to detect, locate and evaluate external events. However, it is not an inherent capacity, and normally develops in early life as the brain gathers experience with cross-modal cues. Thus, depriving it of visual-auditory experience (e.g., by rearing in darkness, omnidirectional masking noise, or in the absence of concordant visual-auditory cues) precludes the development of this neural process and its behavioral sequelae (see companion poster). The present study examined whether rehabilitative training with visual-auditory cues in compromised adult cats would promote development of this capability and its normal performance benefits in detection/localization tasks. Three cats, reared to adulthood in omnidirectional noise or darkness, were trained to approach brief (50 ms) visual or auditory cues that appeared at random locations in a perimetry apparatus. Unlike normal controls, they showed no performance benefits in detection and/or localization to the cross-modal combination anywhere in space. They were then provided with rehabilitative training (repeated exposures to spatiotemporal concordant visual-auditory cues) at a single location (45° of eccentricity in the right hemifield). The rehabilitative effects were striking. Animals now showed normal multisensory performance benefits but these were localized to the training location and its immediate surround. The site-specificity of this multisensory behavioral benefit closely parallels the neural correlates previously observed in single superior colliculus (SC) neurons after similar rearing and training conditions (see Yu et al. 2010), thereby underscoring the close neural-behavioral correlates in these SC-mediated behaviors. These results also indicate that sensory training paradigms represent a valuable therapeutic option for human patients with

similar anomalies of sensory development (e.g., patients with congenital cataracts later corrected), and can also explain variation in the multisensory capabilities of these patient populations after their exposure to sensory-rich environments. Supported by NIH R01 EY031532 and the Tab Williams Family Foundation.

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Poster

636. Cross-Modal Processing II

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Program #/Poster #: 636.17

Topic: D.08. Multisensory Integration

Support: NIH Grant EY026916
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Title: Rehabilitation of hemianopia following stroke with multisensory training

Authors: *B. A. ROWLAND, B. E. STEIN;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Hemianopia (blindness on one side of space) is a debilitating disorder commonly resulting from unilateral damage to visual cortex. We have recently demonstrated the effectiveness of a multisensory training paradigm in rehabilitating hemianopia using an animal model. In the present study, this technique was tested in two patients rendered hemianopic via stroke. Patients were confirmed to have a stable hemianopia lasting for at least 6 months. They participated in ten weekly sessions in which they were exposed to multiple (~600 trials/session) visual-auditory stimulus pairs in the blinded hemifield using a custom apparatus. The results were dramatic and resembled the pattern previously observed in the animal model. Within a few sessions, both patients regained the ability to detect, localize, and report on visual stimuli in their formerly blinded field while maintaining central fixation. Recovery was first initiated at the margin of their sighted field and progressively expanded to include locations far in the periphery. They were able to detect visual stimuli in the recovered field even when another light was presented contralaterally. Both patients reported significant improvements in quality of life, including improvements in navigation and reading. The results were consistent with a model in which spatiotemporally congruent visual-auditory stimulation engages multisensory plasticity in subcortical-cortical networks and amplifies sensitivity to remaining visual inputs after cortical damage. Because the multisensory training paradigm works rapidly and requires little effort, it represents a valid therapeutic paradigm for restoring visual function after stroke. Supported by NIH R01 EY026916 and a pilot grant from the Wake Forest School of Medicine Clinical Trials and Innovation Center.

Disclosures: B.A. Rowland: None. **B.E. Stein:** None.

Poster

636. Cross-Modal Processing II

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Topic: D.08. Multisensory Integration

Support: NIH Grant R01 EY031532
Tab Williams Family Foundation

Title: Multisensory behavior: competition precedes cooperation

Authors: *B. E. STEIN, S. A. SMYRE, B. A. ROWLAND;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The brain has a powerful ability to integrate congruent signals across multiple sensory modalities to enhance perception and behavior. This phenomenon of multisensory integration has been extensively studied in the neurons of the superior colliculus (SC) and the detection and localization behaviors they mediate. The integration of spatiotemporally congruent visual-auditory stimuli produces robust response enhancement in SC neurons and similarly impressive enhancements in the animals' ability to detect and localize stimuli. However, this ability is neither innate nor genetically prescribed. When reared without requisite multisensory experience, SC neurons retain their default multisensory computation, which we have hypothesized is a competition between the senses (Yu et al., 2018). The present study evaluated whether this competition at the neural level would also be observed in behavior. "Naïve" animals reared without visual-auditory experience (either by being reared in the dark [N=1] or omnidirectional sound [N=3]), whose SC neurons do not integrate visual-auditory stimuli to enhance responses, were tested in a detection/localization task with visual, auditory, and visual-auditory stimuli with varying amounts of spatial concordance. Their behavior was contrasted with that of normal controls. As predicted, spatiotemporally congruent visual-auditory stimuli failed to elicit behavioral response enhancement in naïve-reared animals. Tests involving spatially disparate stimuli gave greater insight into the underlying computation. These tests revealed the presence of an unbalanced competition in which each animal's preferred modality dominated, effectively suppressing, the signals from the opposite modality when both were simultaneously stimulated. Animals' preferences were also as expected: the preferred modality was audition for dark-reared animals, and vision for noise-reared animals. This finding has significant implications for our understanding of the default state of the multisensory brain: one in which signals from different sources are processed as mutually competitive, even when the information they offer is objectively congruent. It is only through encoding experience with cross-modal stimuli that the brain develops its typically robust multisensory integration capabilities. In this way, the computation of competition proceeds a computation that is cooperative. Supported by NIH/REI R01 EY031532 and the Tab Williams Family Foundation.

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Poster

636. Cross-Modal Processing II

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Topic: D.08. Multisensory Integration

Support: EY031532
Tab Williams Family Foundation

Title: Asymmetric Hearing Loss Impacts Auditory Localization and Multisensory Integration

Authors: *N. L. BEAN, J. E. NOFZIGER, B. E. STEIN, B. A. ROWLAND;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The brain integrates information from multiple sensory modalities to enhance its ability to detect and localize environmental events. The products of multisensory integration are greatest when the sensory-specific signals being integrated are weak. This process is also dependent on spatial alignment: congruent cross-modal stimuli elicit the largest enhancements, while incongruency depresses responses. Disorders of sensory processing can affect the strength of signals and/or the congruence of cross-modal information, producing different predictions for how they alter multisensory processing. Here we studied multisensory detection/localization behaviors in an animal (cat) model of acute asymmetric hearing loss (ASHL). ASHL is known to both degrade the fidelity of the auditory modality and disrupt normal congruency within the superior colliculus (SC), a structure previously linked to multisensory enhancement in this behavior. Cats were trained to detect/localize and approach brief (50 ms) visual and auditory (broadband) stimuli at multiple locations in a perimetry apparatus. They were then tested with a battery of visual and auditory stimuli (broadband, low-pass filtered, high-pass filtered) presented alone or together in congruence at either full or reduced intensities. Multisensory enhancements in behavioral performance were significant when the intensity of the individual stimuli were reduced (i.e., signals were weaker). Animals were then acclimated to wearing a fitted earmuff over one ear to simulate ASHL, and retested with reduced-intensity visual stimuli, full-intensity auditory stimuli, and their congruent combinations. The earmuff significantly reduced auditory-alone performance to levels similar to that observed with intensity reduction; however, it also significantly reduced multisensory enhancement in contralateral space and eliminated it in ipsilateral space. Detailed analysis showed that the absence of enhancement was largely attributable to a disruption in the ability of animals to accurately localize the auditory stimulus in the ASHL condition. The results were similar across the multiple auditory stimuli tested. These findings suggest that ASHL significantly impairs multisensory processing in detection/localization in ways that are commonly unappreciated, but predictable from its effects on the physiology of midbrain circuits that support these behaviors. This animal model can be useful in exploring the behavioral and electrophysiological properties, relevant plasticity, and rehabilitative options relevant to this disorder. Supported by R01 EY031532 and the Tab Williams Family Foundation.

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Poster

636. Cross-Modal Processing II

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NWO VENI (VI.Veni.192.231)

Title: Reconsidering the old with the new: Objective segmentation of cortical layers and subdivisions of the insular cortex using Nissl histology and deep learning

Authors: *M. A. MUNIAK[1], B. C. JONGBLOETS[1,2], Y. CHEN[1], I. K. GINGERICH[1], T. MAO[1];
[1]Vollum Inst., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Cellbiology, Neurobio. and Biophysics; Biol. Dept., Fac. of Science; Utrecht Univ., Utrecht, Netherlands

Abstract: The advent of histological cell staining techniques (e.g., the Nissl method) enabled neuroanatomists to describe the structure of the brain on the basis of cytoarchitectonics—the size, shape, and arrangement of its cellular components—setting the foundation of modern neuroscience. For example, the laminar organization of the cerebral cortex—the characteristic distribution and morphologies of different neurons between the pia and white matter—stands as a fundamental cornerstone upon which much of our understanding of cortical structure and function is framed. However, despite over a century of careful examination, laminar demarcations remain largely reliant on the trained anatomist's eye, with boundaries being conspicuous in most, but not all, cases. Furthermore, with our ability to collect neuroanatomical and functional data continuing to rise exponentially, the manual annotation of histological sections is becoming increasingly intractable just as quantitively mapping brains from functional experiments to anatomical features is becoming increasingly important. To address these needs, we developed a scalable analysis pipeline which facilitates the objective identification of laminar boundaries in Nissl-stained sections. We first leverage the power of Cellpose (Stringer et al., 2021), a recently developed generalist, deep learning-based segmentation algorithm, to identify the positions and approximate sizes of millions of cells across serial sections of the mouse cortex. Local variations in average soma size and density are then analyzed with respect to a "locally correct" flatmap. The results reveal clear layering patterns whose bounds are extracted using standard edge detection techniques. To demonstrate this pipeline can be used as a high-throughput approach for quantitative analyses of laminar definition, particularly in non-primary cortices, we assessed the mouse insular cortex, which is split into granular, dysgranular, and agranular (AI) cortices on the basis of the presence or absence of a cortical layer IV. Our

technique readily captured this vanishing layer, and also highlighted relatively large cells in layer V as a characteristic feature of the AI, allowing for clear demarcation of these subregions. Furthermore, our analyses revealed variations in relative laminar thickness throughout the insula, even within the same subregion. We anticipate this ability to objectively capture information about cortical layers from histological sections will greatly enhance the contextualization of neuronal data from complementary functional modalities, as highlighted in several projects from our lab presented at this meeting.

Disclosures: M.A. Muniak: None. **B.C. Jongbloets:** None. **Y. Chen:** None. **I.K. Gingerich:** None. **T. Mao:** None.

Poster

636. Cross-Modal Processing II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 636.21

Topic: D.08. Multisensory Integration

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BRAIN Initiative R01 (NS104944)
NWO VENI (VI.Veni.192231) to BCJ

Title: Origin-specific integration of long-range cortical inputs to agranular insular pyramidal neurons

Authors: *I. K. GINGERICH[1], M. A. MUNIAK[1], Y. CHEN[1], B. C. JONGBLOETS[2], T. MAO[1];
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Abstract: Origin-specific integration of long-range cortical inputs to agranular insular pyramidal neurons
Ian K Gingerich, Michael A Muniak, Yang Chen, Bart C Jongbloets, Tianyi Mao Vollum Institute, Oregon Health & Science University, Portland, OR 97239 USA
The insular cortex receives inputs from many cortical and subcortical structures, and integrates these inputs to mediate diverse functions. The agranular insula (AI) receives inputs from cortical areas associated with multi-modal sensory and higher order processing, including regions associated with olfactory, visual, motor, and proprioceptive processing. However, how these inputs integrate in the AI and facilitate diverse AI function through different cell types and distinct subcellular circuits remains elusive. Here, we use subcellular ChannelRhodopsin-2 Assisted Circuit Mapping (sCRACM) (Petreanu et al, 2009; Mao et al, 2011) and morphological reconstruction to generate a morpho-subcellular atlas to investigate the functional connectivity

and the subcellular organization of these inputs to the AI. sCRACM allows us to map the spatial distribution of synaptic inputs along the dendrites of recorded neurons at subcellular resolution by expressing ChR2 in presynaptic neurons. Presynaptic axon terminals are excited focally via sequential laser pulses, triggering local neurotransmitter release which, in combination with extracellular application of Na^+- and K^+- channel blockers, results in robust excitatory postsynaptic currents ($EPSC_{sCRACM}$). This technique is well suited to the investigation of long-range inputs, in which the presynaptic somata may reside in a different brain region than that of the recorded neuron.
To investigate cortical input organization, we systematically injected ChR2 in the candidate cortical regions that send projections to the AI based on recent anatomical studies (Gehrlach et al, 2020; Deng et al, 2021). We performed sCRACM experiments by recording from pyramidal cells located in all layers of the AI. Analysis of sCRACM maps identified insular region-specific, layer-specific, and dendritic-compartment-specific input patterns. In conjunction with morphological analysis of the recorded pyramidal neurons, we conclude that cell type specificity and unique patterns associated with each input source are some of the ways by which the insular cortex may mediate its diverse functions.

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Poster

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Title: Local circuits of the insular cortex differentially integrate long-range connections

Authors: *Y. CHEN[1], B. C. JONGBLOETS[2], M. A. MUNIAK[1], I. K. GINGERICH[1], T. MAO[1];
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Abstract: The insular cortex (IC) is an information-integration hub that mediates diverse functions such as decision making, taste perception, and interoception. While great progress has been made using in vivo optogenetics, pharmacology, and calcium imaging to establish distinct

functional roles of the anterior and posterior subregions of the IC, their underlying circuit mechanisms remain elusive. To better understand how local signal processing within the IC contributes to its diverse functions, our study aimed to decipher IC local circuits that are associated with specific input sources and output targets.
To comprehensively identify local inputs to specific IC output neurons, we utilized laser scanning photostimulation (LSPS) with caged glutamate, coupled with patch-clamp recordings of neurons labeled with retrograde bead injections in either amygdalar subregions, thalamic subregions, or the contralateral IC, known major projection targets of the IC. We found that excitatory input patterns to IC pyramidal neurons exhibit stereotypical distributions across cortical depths. Importantly, these local input patterns—often differing from the canonical microcircuits observed in primary sensory cortices—are unique based on IC projection targets. Inspired by the projection-specific local input patterns, we uncovered an information flow pathway within the IC that mediates the integration of posteriorly-arriving long-range inputs to anteriorly-located IC output neurons. Although the anterior and posterior subregions of the IC have been extensively reported to mediate distinct functions, our results provide the possibility that they may be synergistically recruited during certain behaviors, thereby providing a new perspective on how functional diversity within the IC can be achieved.
Together, our findings show that local circuits in the insular cortex are specifically tailored to integrate unique neuronal signals for different input and output pathways, which elucidates the neuronal substrates responsible for information-integration within the IC.

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Poster

636. Cross-Modal Processing II

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NWO VENI (VI.Veni.192.231)

Title: Principal cell types of the mouse insular cortex underline its functional diversity

Authors: *B. C. JONGBLOETS[1,2], Y. CHEN[2], I. K. GINGERICH[2], M. A. MUNIAK[2], T. MAO[2];
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Abstract: The insular cortex is a critical multi-modal integration site and is pivotal for cognitive functions, homeostatic state and motivational processing, and interoceptive awareness. The insula carries out these processed state signals to particular amygdalar, thalamic, and cortical subregions. Recent *in vivo* calcium imaging studies have identified that the activities of subpopulations of insular neurons correlate with specific behaviors. In addition, anatomical tracing studies indicate that input to and outputs from the insula have a specific topolographic organization, suggesting the insula is composed of specialized local neuronal circuits with distinct cell subtypes. Yet, a comprehensive understanding of the excitatory cell types in the insular cortex is currently lacking. Cell type classifications based on other cortical areas do not necessarily apply to the insula due to its unique cytoarchitectural structure, including the graded presence of the cortical layer 4.
In this study, we performed whole-cell patch-clamp recordings of the pyramidal neurons in the insular cortex of adult mice using acute brain slice preparations. Electric measurements of passive and active properties, including membrane resistance, action potential waveform, and spike behavior were obtained under current-clamp configuration with biocytin-filled pipettes. Dendritic morphologies of the recorded neurons were then revealed using post-hoc biocytin staining and reconstruction. Cell type classification was performed based on a dataset of over 900 reconstructed dendritic morphologies and 300 electrical profiles. Anatomical locations of the cells were also tracked. In addition, cortical, thalamic, and amygdalar projection targets for over 250 recorded pyramidal neurons were identified using retrograde anatomical tracers. Based on the above-mentioned parameters, we identified putative morphological, electrical, and cross-modality cell types. Interestingly, several cell types are located at specific insular subregions and project to distinct targets, indicating that local specializations of the neuronal circuits are in place to accommodate multi-modal information processing. To enable linking the future functional experiments directly to cell types identified here, we trained random forest classifiers to identify the proposed cell types based on a short list of features. The trained classifiers allow for further study of the roles of cell types in insular information processing. The utilization of this dataset and the classifiers to investigate insular microcircuits will be showcased in several studies from our laboratory during this meeting.

Disclosures: B.C. Jongbloets: None. **Y. Chen:** None. **I.K. Gingerich:** None. **M.A. Muniak:** None. **T. Mao:** None.

Poster

637. Visual Behavior

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Program #/Poster #: 637.01

Topic: E.01. Eye Movements

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CIFAR

Title: Assessment of remote eye tracking using a video game menu navigation task

Authors: *S. A. STONE[1], C. S. CHAPMAN[2];
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Abstract: Eye tracking has become more cost effective and portable. It is now possible to perform eye tracking research outside of the lab using monitor-mounted systems or webcams. Studies have demonstrated that monitor-mounted eye trackers have low spatial error and high sampling rates, making them suitable for laboratory data collection. On the other hand, webcams tend to be more variable; different resolutions, quality, and frame rates can lead to vastly lower spatial accuracy and sampling rates. Good video game design necessitates intuitive menu design. We created a simple menu navigation task that mimics the game menu system of a popular video game (Mass Effect 3) to compare monitor-mounted versus webcam eye tracking. The participants were presented with a prompt, which directed them to complete a simple task (e.g. “Turn on the subtitles.”). During the task, the participant’s mouse and gaze positions were recorded. The task was assessed in two studies: one in-person and one remote. The first study simultaneously assessed monitor-mounted (Tobii Eyetracker 4C) and webcam (Labvanced) eye trackers on employees from a well-known video game company. The second study investigated the feasibility of deploying the same task remotely using the MTurk recruitment platform and Labvanced webcam eye tracking. Dependent measures were extracted from the dataset to calculate four broad metrics: time, mouse movements, gaze behaviour, and hand-eye coordination. We analyzed data from the first study with the goal of uncovering significant differences of performance between the monitor-mounted and webcam eye tracker. Task performance differences were calculated between the subject pools. The webcam eye tracker performs similarly to the monitor-mounted solution, suggesting that remote eye tracking studies are feasible. Our analysis also uncovered a previously unknown “friction point” (i.e. unintended difficulty) in the menu system’s design - users appeared to be unable to find one of the menu options effectively. This was replicated in both studies, demonstrating the webcam eye tracker is sensitive enough to detect user confusion. However, it is important to note that webcam eye tracking will have a lower spatial resolution and collection frequency — limitations that can exclude certain experimental designs. We conclude that webcam eye tracking systems are practicable if the experimenter adheres to basic principles that account for the decreased sampling rate and accuracy inherent to webcam eye tracking.

Disclosures: S.A. Stone: None. **C.S. Chapman:** None.

Poster

637. Visual Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 637.02

Topic: E.01. Eye Movements

Title: The functional coupling of eyeblinks and car control during Formula car driving

Authors: *R. NISHIZONO, N. SAIJO, M. KASHINO;
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Abstract: Vehicle drivers effectively use gaze strategies to collect visual information for trajectory planning and visual guidance. However, little is known about whether natural occlusion, i.e., eyeblinks, are modulated to avoid missing critical information. It seems likely that driver's eyeblinks will be biased toward less critical spatio-temporal timings. To test this hypothesis, we determined Formula car driving as an extreme driving situation, in which the cost of misjudgment is maximally emphasized, and elite drivers will have exact and stable internal memory of the courses. Three professional Formula car drivers participated in the study, conducted in three courses, during free practice before racing and dedicated car testing sessions. We simultaneously recorded eye images and car control behavior.First, we extracted the eyeblink timings and analyzed the relationship between the timings and the car behavior. The results revealed that eyeblinks were coupled with the locations on the course. These geographic eyeblink patterns consistently appeared in every driver and circuit. While eyeblink rates during a lap were not significantly correlated with lap driving performance, eyeblink synchronicity among laps was consistently higher for faster laps, indicating that the eyeblink patterns converged as lap performance got higher. Next, we analyzed the acceleration patterns for the eyeblink timings. The results revealed that eyeblinks were suppressed during longitudinal deceleration and higher lateral acceleration, consistent with the higher weight of visual information during the early cornering phase. A generalized linear model was applied to study eyeblink generation probability according to the driver, acceleration, and lap time performance. The results revealed that each individual variable and the interaction terms between driver and acceleration as well as between acceleration and lap time were significant, indicating that the engagement level for driving changes the perceived cost of eyeblinks.These results suggest that eyeblinks are modulated with effective strategies for minimizing uncertainty during race driving, and the strategies are sensitive to the changes in within-individual engagement.

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Poster

637. Visual Behavior

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Topic: E.01. Eye Movements

Support: MEXT Grant 20H04286
MEXT Grant 18KK0286
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Title: Humans track the falling objects more accurately than the rising objects irrespective of acceleration conditions and vestibular gravity information

Authors: T. HIRATA[1,2], ***Y. HIRATA**[3,4,5], N. KAWAI[1,4];
[1]Cognitive and Psychological Sci., Nagoya Univ. college of Informatics, Aichi, Japan; [2]Res. Fellow of Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Chubu Univ. Col. of Engin., Chubu Univ. Col. of Engin., Aichi, Japan; [4]Chubu Univ. Acad. of Emerging Sci., Aichi, Japan; [5]Chubu Univ. Ctr. for Mathematical Sci. and Artificial Intelligence, Aichi, Japan

Abstract: Motion of any objects on the earth is under the influence of gravity. A previous study in the virtual reality (VR) environment showed that humans accurately estimated a position of the downward moving object at 1G (9.81 m/s^2) acceleration condition compared to that of upward moving object, suggesting that humans can accurately track free fall objects. This superior recognition for free fall object can attain by some reasons which derive from our "natural" environments: 1) the direction of the free-fall visual object corresponded to the actual gravitational direction, 2) the speed of visual object was of gravity acceleration. If the correspondence between visual direction of the moving object and gravity direction sensed by vestibular system plays a role, then the superior recognition will not be found by a supine posture. If humans are sensitive to a downward (falling) motion at 1G acceleration, then the superior recognition will not be found at other acceleration conditions such as a constant speed (0 acceleration). We examined these possibilities as well as confirmed the superior recognition of a free fall object against a "free rise" object in Experiment 1. We explored a potential role of the smooth pursuit eye movement ability for free fall objects in Experiment 2. Twenty-three participants with a Head Mount Display watched a white ball (visual angle: 0.8 deg) on a dark background moved either from upper to lower or from lower to upper at 1G (acceleration), -1G (deceleration) or 0G (constant velocity) acceleration conditions. Participants were instructed to press a button at the timing when the ball pass through a ring-shaped goal either in upright or supine posture. We evaluated the timing difference between the actual timing of the ball and that of key press. The position of the falling ball was more accurately recognized than that of the rising ball at 1G and 0G acceleration conditions, while the opposite was the case at -1G deceleration condition in both postures, indicating that the superior recognition for free fall objects is irrelevant to vestibular information (actual gravity direction) at least in VR situation. In Experiment 2, 36 participants followed the same ball motions either at 1G or 0G condition either in upright or supine posture. We evaluated the difference between the actual ball position and its gazed position. The gaze position was more accurate for the falling balls than the rising balls in both acceleration conditions irrespective of the postures, suggesting that the superior recognition for free fall objects can attribute to the superior eye tracking ability, which is, again, irrelevant to vestibular information (actual gravity direction).

Disclosures: T. Hirata: None. **Y. Hirata:** None. **N. Kawai:** None.

Poster

637. Visual Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 637.04

Topic: E.01. Eye Movements

Title: Altered visual strategy during the Trail Making Test in adults with versus without ADHD

Authors: K. ANDERSEN[1], ***K. LANDWEHR**[1], M. SPINETTA[2], B. HEINTZ WALTERS[1]; [1]Dept. of Kinesiology, [2]Dept. of Psychology, Seattle Univ., Seattle, WA

Abstract: Introduction. Attention-deficit hyperactivity disorder (ADHD) is a neurological disorder characterized by impulsiveness and changes in attentional processes that negatively impact quality of life. Currently, no established, objective diagnostic assessment for adult ADHD exists. Eye movements are closely linked to attentional processes and the assessment of eye movements could potentially serve as an objective diagnostic tool for ADHD in adults. The purpose of this study was to examine eye movements during a commonly used neurophysiological assessment in adults with and without ADHD. **Methods.** Eye movements were recorded from 13 adults with (age 18 - 38 years; 8 female, 5 male) and 13 adults without (age 19 - 24 years; 6 female, 7 male) ADHD while performing the Trail Making Test, a commonly used neurophysiological assessment of attentional processes. Participants completed the Trail Making Test displayed on a touchscreen under three conditions: 1) Trail Making Test A, 2) Trail Making Test B, and 3) Trail Making Test B while performing a visuospatial dual task. Performance on a standardized test of attention (The Test of Everyday Attention) was also measured. **Results.** All participants used a series of saccades and fixations during the Trail Making Tests. Adults with ADHD made more saccades than adults without ADHD for Trail Making Test B while performing the visuospatial task (2.00 ± 0.35 and 1.67 ± 0.14 saccades per second, respectively). There was no difference in saccade number between groups for Trail Making Test A and Trail Making Test B. Increased saccade number was related to decreased performance on the Test of Everyday Attention in adults with ADHD ($R^2 = 0.434$). **Conclusions.** Differences in eye movements between adults with and without ADHD reveal an altered visual strategy in adults with ADHD while performing the Trail Making Test with a dual-task. A relationship between saccade number and performance on a standardized measure of attention supports the idea that eye movements are associated with attentional processes in this population. Future work could continue to examine eye movements in adults with ADHD and whether they could serve as an objective diagnostic tool in this population.

Disclosures: K. Andersen: None. **K. Landwehr:** None. **M. Spinetta:** None. **B. Heintz Walters:** None.

Poster

637. Visual Behavior

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Program #/Poster #: 637.05

Topic: E.01. Eye Movements

Support: MITACS
NSERC DG

Title: Dynamics of eye-hand coordination are flexibly preserved in eye-mouse coordination during an online, screen-based interaction task

Authors: *J. K. BERTRAND, C. S. CHAPMAN;
Univ. of Alberta, Edmonton, AB, Canada

Abstract: We investigated whether computer-mouse/eye-gaze dynamics resemble real world hand/eye coordination. We used an online, eye-tracking-enabled platform (Labvanced; Finger et al., 2017) to create a screen based version of a real world movement task (Lavoie et al., 2018). Instead of moving cups to targets on a table, crowdsourced participants dragged circles to targets on their computer screen. Movements followed a specific sequence while we recorded mouse movements and webcam eye gaze. Participants watched an instructional video and then had at least 2 guided practice trials with feedback. During the 50 self-paced experimental trials, no feedback was given. To encourage high quality eye gaze data, we 1) employed a virtual chinrest; 2) had participants complete a 5-minute initial calibration; 3) recalibrated every 5 trials (10 times) throughout the task; and 4) required stable internet and certain webcam/technology. The eye gaze data were rigorously cleaned, including k-means clustering to bin the data into meaningful areas of interest. Trials were segmented into 8 distinct circle-dragging movements, each with a Pick-up, Drag, and Drop-off phase. Of 51 datasets, 16 had unusable eye data and 6 had extensive sequencing errors, thus, 29 datasets were analyzed.
At Pick-up, we found similar patterns between mouse/gaze coordination and real world hand/eye coordination (Lavoie et al., 2018). Notably, eye gaze arrived at the Pick-up location nearly 500 ms before the mouse, and left < 100 ms after Pick-up. However, Drop-off in real and screen worlds differed. Whereas real world eye gaze arrives at the Drop-off location around 500 ms before Drop-off and leaves quickly thereafter, in the screen world, eye gaze arrived at the Drop-off location only 133 ms prior to Drop-off, and continued fixating there for another 540 ms after. Thus, although the overall length of fixation (~500 ms) remained relatively consistent, the majority of the fixation occurred after Drop-off. The earlier arrival to Drop-off is in some sense required since the circle-dragging action (~217 ms) is not long enough for a 500 ms head start. What is notable is that the earlier arrival does not engender decreased fixation length, which instead persists for ~500 ms. We propose that this flexible but enduring fixation behaviour may be a baseline cognitive requirement for both digital and real object interactions.

Disclosures: **J.K. Bertrand:** None. **C.S. Chapman:** None.

Poster

637. Visual Behavior

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Topic: E.01. Eye Movements

Support: NIH R01-EY025648
NSF 1848939

Title: Dynamic saccade context triggers more stable object-location binding

Authors: *Z. LU, J. D. GOLOMB;
Dept. of Psychology, The Ohio State Univ., Columbus, OH

Abstract: Despite receiving visual inputs based on eye-centered (retinotopic) coordinates, we are able to perceive the world-centered (spatiotopic) locations of objects. A long-standing debate has been how object representations are transferred from retinotopic to spatiotopic coordinates to achieve stable visual perception across eye movements. Many studies have found retinotopic effects even for higher level visual processes, like object-location binding. However, these studies often rely on fairly static contexts (prolonged fixation on one location, followed by a single saccade). Might spatiotopic object-location binding be triggered selectively in dynamic saccade contexts? To test this hypothesis, we modified the behavioral 'spatial congruency bias' (SCB) paradigm. Human participants had to judge if two objects presented sequentially were the same or different. We conducted four experiments to investigate retinotopic vs spatiotopic object-location binding in different states. Critically, we found both strong spatiotopic and retinotopic SCBs in a dynamic saccade context with continuous eye-movements (Expt 1), but only retinotopic in the static context with only one saccade (Expt 2). Expts 3 and 4 isolated different dynamic saccade factors and showed that repeated saccades could decrease the retinotopic bias, and having the stimulus present before, during, and after the eye movement could increase the spatiotopic bias. Thus, using a consistent experimental design, we isolated factors that directly change object-location binding from retinotopic to spatiotopic. Based on these results, we propose an object location mapping theory with a dynamic state and gating model, which provides a possible interpretation to help us intuitively understand how our brains represent object location across saccades. Overall, our results provide strong evidence that dynamic saccade context can trigger an integrative and dynamic brain state to form more stable object-location binding, which is crucial to improved understanding of how the brain achieves visual stability in the dynamic world.

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Poster

637. Visual Behavior

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Title: Human gaze behaviour linked with image structure that discriminates perceived materials

Authors: *A. C. SCHMID, M. NAU, C. I. BAKER;
Lab. Brain and Cognition, NIH, Bethesda, MD

Abstract: Accurately recognising materials and their intrinsic properties from visual information is vital for successful interactions with our environment, from avoiding slippery floors to handling fragile objects. The perceived material of an object is therefore inherently linked with its behavioral affordances. Moreover, the way we interact with an object also affects how we visually sample it, as our gaze is guided to behaviorally relevant features. We hypothesized that human gaze behavior during object viewing should therefore be guided by the object's perceived material and, if so, visual sampling of the object should reflect regularities in image structure that perceptually define the material. To test this, we characterised the relationship between human gaze behaviour, image structure, and material perception by combining eye tracking and deep-learning-based gaze predictions for 924 rendered photorealistic object stimuli. These stimuli were complex glossy objects rendered in natural illumination fields with varying reflectance properties, leading to a wide range of material appearances such as plastic, clay, ceramics, fabric, etc. Using DeepGaze IIE to predict fixation patterns on these images, we found that these patterns do indeed differ between stimuli, independent of object shape. This suggests that surface properties affect how we visually sample objects. Further, these differences in gaze patterns correlated with differences in perceived material. Finally, we found that variations in contrast, clarity, size, and colour of specular reflections of the objects predicted differences in both perceived material and gaze behaviour, providing a direct link between image cues, viewing behaviour, and affordance-related information. In a series of follow-up analyses, we then tested these model-based predictions in eye tracking data. Collectively, our results support the notion that both object perception and viewing behaviour are shaped by the affordances of the things we look at, and that both can be linked to regularities in image structure caused by the complex yet characteristic ways that light is scattered by materials with different surface properties.

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Poster

637. Visual Behavior

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Program #/Poster #: 637.08

Topic: E.01. Eye Movements

Support: Dartmouth College, Department of Psychological and Brain Sciences

Title: Saccadic Localization of Illusory Position

Authors: *S. SALEKI[1], P. CAVANAGH[3], P. U. TSE[2];
[2]Psychological and Brain Sci., [1]Dartmouth Col., Hanover, NH; [3]Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: Visual perception of object position is influenced by moving reference frames. For example, a fixed point on a rolling circle appears to have a circular motion, while its actual motion trajectory traces a cycloid. Recently, it has been shown that a pair of transient probes flashed in alternation within a moving frame show large position displacements (Frame Induced Position Shift, or FIPS), such that perceived position of the probes corresponds to their location with reference to the frame. This results in a dramatic perceptual shift that can be as large as the displacement of the moving frame. Here, we asked whether saccadic programming is also influenced by FIPS when participants try to visually localize the probes. We collected data in separate tasks with probes that flashed for either short or long durations to measure the magnitude of FIPS as well as the amplitude of saccades. In the perceptual tasks, participants reported the amount of the illusory displacement and in the saccade task, they were instructed to shift their gaze from a fixation point to the position of a cued probe inside the frame. Participants reported perceptual frame effects from 50% to 100% of the frame's displacement. Results from the saccade task show that the landing positions of saccades are shifted in the direction of the illusory displacement by 10 to 20% of the frame's displacement. This effect is similar for short and long duration probes, but smaller in magnitude compared to the displacement reported in the perceptual tasks. Together, our data suggest that when directing gaze to briefly presented stimuli, saccades to a target are biased by a moving frame, but to a lesser extent than perception is.

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Poster

637. Visual Behavior

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Program #/Poster #: 637.09

Topic: E.01. Eye Movements

Title: Operationalizing situational awareness in policing: an eye-tracking study

Authors: *P. M. DI NOTA[1], J.-M. HUHTA[2], E. ROPO[3], J. P. ANDERSEN[4];
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Abstract: Situational awareness (SA) is the most important skill required by police and law enforcement officers, allowing them to effectively assess and respond to public encounters including critical incidents. SA has traditionally been defined as a combination of neuropsychological processes, namely perception of the surrounding environment, understanding how various cues provide relevant information, and prediction of how the situation can develop. Incomplete or sub-optimal SA strategies, including a lack of updating visual information, can lead to errors in subsequent judgement, decision-making, and action, including use of force (UOF). Errors in the selection and application of UOF, especially lethal force, in training or operational field settings have severe consequences for learning, occupational health, and public

safety. Therefore, adequately defining, operationalizing, and instructing SA in police-specific contexts is an important gap to fill in existing applied literature and practice. Utilizing eye-tracking technology, the current cross-sectional study aimed to track the development of early-stage SA (i.e., visual perception) in early (n=10, 6 female, Mage=25.6) and intermediate (n=13, 5 female, Mage=24.6) novice police trainees as well as experienced police officers and instructors in tactics and UOF (n=11, 0 female, Mage=41.6) from Finland's Police University College. Participants' fixation order and visit duration (seconds) were tracked while they viewed 13 static images of various staged encounters, ranging from non-threatening to high threat. Interviews were conducted between each image probing what cues officers perceived and how they would respond. Quantitative analyses revealed that, in the first 5 seconds of stimulus onset, all participants fixated earlier on suspects' faces compared to hands, bodies, or the peripheral environment ($p<0.001$). However, early novices fixated significantly later on suspect persons' hands compared to both intermediate novices ($p=0.008$) that had completed their UOF and tactical training, and experienced officers ($p=0.029$) who spent less time scanning the environment than both novice groups ($p<0.05$). The current findings provide novel empirical insight unto the neurophysiological mechanisms underlying a critical occupational skill for police and law enforcement. Using objective measures of visuomotor behaviour to derive tacit expert knowledge, the current findings are being used to inform and develop evidence-based police training in SA, UOF, and tactics, contributing to increased occupational health and public safety.

Disclosures: P.M. Di Nota: None. **J. Huhta:** None. **E. Ropo:** None. **J.P. Andersen:** None.

Poster

637. Visual Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 637.10

Topic: E.01. Eye Movements

Support: JST Grant Number JPMJSP2124
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Title: Properties of fast and smooth vergence eye movements in baseball players

Authors: *Y. YOSHIMURA[1], T. KIZUKA[2], S. ONO[3];
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Abstract: In baseball, it is important to acquire information about visual motion in depth. For example, baseball fielders are required to accurately hit or catch a ball moving from far to near. Thus, the ability to acquire beneficial visual information in depth could be associated with effective performance in baseball. Vergence eye movements are one way to acquire depth

information and are disconjugate eye movements in which the eyes move in opposite directions so that the image of a single object is held simultaneously on the fovea of each eye. Vergence eye movements are classified into convergence and divergence eye movements, which are induced by visual stimuli moving from far to near and from near to far, respectively. It is also important for baseball players to fast gaze at and smoothly pursue a visual target in depth using fast and smooth vergence eye movements. These eye movements allow us to continuously hold the target on the fovea to acquire apparent and accurate visual information in depth. However, the properties of fast and smooth vergence eye movements in athletes are still uncertain. Therefore, the main purpose of our study was to reveal the difference in fast and smooth vergence eye movements between baseball players and non-athletes. We used the green laser with a diameter of 2 mm as the visual target for two experimental oculomotor tasks. One task was a fast vergence task where a visual target randomly moved at three different positions in depth. Participants were asked to shift their gaze at the visual target as soon as possible. The other was a smooth vergence task where a visual target randomly moved at three different velocities in depth. Participants were asked to pursue the visual target as accurately as possible. The results showed that the latency of fast convergence eye movements in baseball players was significantly shorter than in non-athletes. The results also showed that the initial velocity of smooth convergence eye movements in baseball players was significantly larger than in non-athletes. However, there was no difference in fast and smooth divergence eye movements between baseball players and non-athletes. Therefore, our findings suggest that the superior ability of convergence eye movements in baseball players could be attributed to their abundant experience and motor learning in visually tracking fast-moving targets from far to near.

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Poster

637. Visual Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 637.11

Topic: E.01. Eye Movements

Support: Canadian Institutes of Health Research (MOP-FDN-148418)

Title: Age, gender, and task modulation of eye blink behaviour in humans

Authors: *I. C. PITIGOI, B. C. COE, R. YEP, H. C. RIEK, J. E. PERKINS, R. H. KIRKPATRICK, B. J. WHITE, D. C. BRIEN, D. P. MUNOZ;
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Abstract: Spontaneous blinking of the eyes is an essential physiological behaviour responsible for lubricating the cornea and protecting the eye from foreign particles. However, humans blink at a much higher rate than necessary for these purposes alone, suggesting that higher level cognitive, social, or environmental factors may be involved. Blinks occur at implicit breakpoints

in a task and are sensitive to internal mental states related to attention and cognitive demand. Here, we aim to characterize eye blink behaviour in healthy individuals performing different tasks: a structured interleaved pro- and anti-saccade task (IPAST) and an unstructured video-viewing task (FreeView). Data was collected from 608 controls spanning the ages of 5-93 years (390 female, 218 male) and analyzed to understand blink timing in relation to task demands. In both tasks, blinks were highly organized, occurring most often at times when visual attention was not critical to the task. For example, participants suppressed eye blinks more often on anti-saccade trials than pro-saccade trials in IPAST, and on highly salient video clips in FreeView. Patterns in blink timing differed between age groups in both tasks, which suggests changes across aging and development, despite blink rate itself not being affected by age. At reproductive age, females had a higher blink rate than males overall, and blink structure in both tasks differed by gender, pointing to potential social or hormonal interactions. These findings provide a fundamental understanding of blink behaviour under different task conditions and describe variability between genders and across lifespan. This is crucial to the discovery of non-invasive ocular biomarkers related to cognition, memory, and motor ability, which would have profound implications for earlier diagnosis and intervention in many neurodegenerative or psychiatric patient groups.

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Poster

637. Visual Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 637.12

Topic: E.01. Eye Movements

Title: Does a single-session of occlusion training improve visual-perceptual skills during an interceptive task in virtual reality?

Authors: *K. BURGER[1], T. DAUTLE[1], E. PRYSE[1], M. AUBANEL[1], M. DALECKI[1], N. KUZNETSOV[1,2];
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Abstract: Occlusion training has been adapted to virtual reality (VR) to overload the visual-perceptual system in a safe and controlled manner. The purpose of this study was to test the effect of occlusion training on interceptive skills learned in a single VR session and whether this mode of training would elicit improvements in visual reaction time (VRT). We hypothesized that the group receiving occlusion training would catch fewer balls through the training session due to the increased difficulty, but that the overload may lead to better catching percentage at retention. This overload may also elicit greater retention of VRT improvements compared to the group receiving no overload during training. Young adults (N = 54, 24 males, M_{age} = 21.2 yrs)

were placed in a virtual football stadium via a head-mounted display (HTC Vive ProEye) and wore trackers on their wrists that rendered their hand position in VR. Once ready, a quarterback in the environment would throw a football and the participant was instructed to catch the ball with both hands. All participants received ten throws for the pre-test, then completed another three blocks of ten throws for training. To test the effects of occlusion, participants in the intervention group trained their catching skills with the ball disappearing for the middle 40% of its trajectory. Catching performance at baseline, during the training session, and at retention (24 hours later) was measured by the percentage of throws caught in each block. Gaze position was recorded throughout each throw with the HTC Vive ProEye, capable of eye-tracking at 90 Hz. Saccade onset, our marker of VRT, was defined as the time when gaze velocity exceeded 150 deg/s. Repeated measures ANOVA was performed to detect within-subject and between-group differences in catching percentage and VRT. A one-way ANOVA was performed to detect differences in retention between groups. Catching skills improved following the training session in VR with respect to baseline (+65%; $p < 0.05$), but in contrast to our hypothesis, improvements were similar between groups ($p>0.05$). On the other hand, VRT remained unchanged and did not show significant differences within subjects over time (baseline: mean 414±310 ms; last training session: mean 423±289 ms, $p>0.05$) or between groups (last training session; controls: mean 456±292 ms; occlusion: mean 460±357 ms, $p>0.05$). These results suggest that virtual catching can be improved with training, but that an overload such as partial occlusion may not induce further advantages. Additionally, a short session of VR training is insufficient to improve VRT. Future studies will explore other training protocols and their effects on skill learning in VR.

Disclosures: K. Burger: None. **T. Dautle:** None. **E. Pryse:** None. **M. Aubanel:** None. **M. Dalecki:** None. **N. Kuznetsov:** None.

Poster

637. Visual Behavior

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Program #/Poster #: 637.13

Topic: E.01. Eye Movements

Support: NIH Grant EY029703

Title: Ambulatory monitoring of ocular alignment with wearable eye trackers to assess the severity of intermittent exotropia

Authors: *J. R. ECONOMIDES, M. D. DILBECK, J. C. HORTON;
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Abstract: Intermittent exotropia affects about 1% of the American population. In this condition, one eye periodically deviates outwards. When fusion is lost, children avoid diplopia by suppressing signals from the peripheral temporal retina in each eye. This suppression blocks the error signal that would normally induce an adjustment in extraocular eye muscle tone to bring

the ocular axes back into alignment. Intermittent exotropia usually remains intermittent, with patients flipping between two states: 1) bifoveal fusion with intact stereopsis versus 2) exotropia with suppression and anomalous retinal correspondence. However, ophthalmologists often recommend surgery because a child's deviation may progress to become constant, resulting in permanent loss of binocular function. There is no consensus regarding the criteria that should guide the decision to perform surgery, or whether the condition in fact progresses inexorably. To address these issues, wearable eyetrackers were used to monitor ocular alignment over the course of the day in 40 patients with intermittent exotropia while they engaged in their regular activities. The eyetrackers recorded the percentage of time spent in an exodeviated state. The prevalence of exotropia ranged from <1% to 93%, with a median of 23%. There was a correlation between the magnitude of exotropia measured in the clinic with the cover/uncover test and measured by the eyetrackers (Pearson r = 0.76). There was also a correlation between patients' (or their parents') estimate of exotropia and the actual prevalence measured by the eyetrackers. However, clinical assessment over-estimated the true prevalence in 33/40 patients, highlighting the need for an instrument to provide accurate, quantitative data. Eye tracking data also showed that a larger magnitude of ocular deviation was correlated (Pearson r = 0.51) with a greater percentage of time that exotropia was manifest. These data provide new insights into the clinical features that characterize intermittent exotropia and will allow clinicians to quantify reliably the percentage of time that the eyes are deviated to inform decisions about optimal approaches to therapeutic management.

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Poster

637. Visual Behavior

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Program #/Poster #: 637.14

Topic: E.01. Eye Movements

Support: Bar Ilan University Gonda Multidisciplinary Brain Research Center student travel encouragement scholarship for international conferences (OK)

Title: Fixation related mismatch negativity and prolonged saccadic inhibition in response to visual oddballs in natural viewing

Authors: *O. KADOSH[1], Y. BONNEH[2];
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Abstract: In natural viewing, the scene is scanned by saccades interleaved by fixational eye movements, which tend to freeze automatically by stimulus presentation. Accumulating evidence shows that such natural viewing conditions could be studied by combining EEG and eye tracking and using saccades as triggers for the event onset of the Fixation-Related Potentials (FRP) and the Oculomotor Inhibition (OMI) that follows every saccade. The result of this analysis was

suggested to be equivalent to flashed stimuli with prior parafoveal preview. Previous studies that measured the response to visual deviants in a sequence of flashed stimuli found increased negativity of the occipital N1 ERP component (visual Mismatch Negativity, vMMN), and prolonged saccadic inhibition for the unexpected. In the current study we examined whether in natural viewing conditions a similar mismatch ERP and prolonged OMI for deviance could be found. We developed a visual oddball paradigm on a static display to generate expectancy and surprise across successive saccades. Observers (N=16), inspected items in specific positions one after the other for 5 seconds per trial. The small patterns E and inverted E were arranged on the screen along a horizontal path with one rare (oddball) and one frequent (standard). Our results show significantly larger FRP of the occipital N1 for the mismatched E compared with the standard and prolonged OMI of the fixational microsaccade as previously found for transient oddballs using abrupt stimulus presentations. Moreover, the latency of microsaccade in the deviant condition, was positively correlated with the amount of preceding standards. Our results show for the first-time prolonged OMI and stronger fixation-related N1 to a visual mismatch in free, but task-guided viewing. These two signals combined could serve as markers of prediction error in future free viewing studies.

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Poster

638. Cerebellum: Non-Motor Functions

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 638.01

Topic: E.02. Cerebellum

Support: NIH Grant UH3NS100543
NIH 5K23NS091344
NIH 1R61AG069729

Title: Attention related neuronal modulation: Evidence of cerebellar mediation in cognition

Authors: *A. SRIVASTAVA[1], R. GOPALAKRISHNAN[2], N. SLOBODIN[1], O. HOGUE[3], M. SCHROEDEL[1], J. BIARS[2], L. F. BOCCA[2], K. B. BAKER[1], A. G. MACHADO[2], D. P. FLODEN[2];
[1]Dept. of Neurosciences, Lerner Res. Inst., [2]Ctr. for Neurolog. Restoration, Neurolog. Inst., [3]Dept. of Quantitative Hlth. Science, Lerner Res. Inst., Cleveland Clin., Cleveland, OH

Abstract: Introduction: The role of Cerebellum in motor control has been well established, however its role in higher order cognitive functions is still poorly understood. Our understanding of the role of cerebellum in cognitive functions comes mostly from studies reporting cognitive deficits in patients with cerebellar damage, as well as neuroimaging and non-invasive stimulation studies. To our knowledge, there is no study which has directly measured human cerebellar activity during cognitive tasks. As a part of the first in-human phase I trial investigating the

effects of deep brain stimulation (DBS) of the cerebellar dentate nucleus (DN) on chronic post-stroke motor rehabilitation, we collected local field potentials from DN and scalp EEG in subjects with middle cerebral artery stroke during a visual attention oddball task. Methods: Nine participants performed a visual oddball task which involves detection of an oddball stimulus (red box) occurring amidst trains of standard stimuli (white boxes) presented on the computer screen. Electrophysiology data from DN and scalp EEG was time-locked to stimulus onset and pre-processed before computing event-related fields at the scalp level and cortico-cerebellar coherence (CCC) to compare standard and oddball trials. Results: Oddball EEG responses showed modulation at central and parietal electrode locations reflecting classical P300 activity occurring 400-600ms post oddball stimulus onset. Decreased CCC was observed in the alpha band after the oddball stimulus onset as compared to standard stimulus. Greater CCC was observed in theta and delta bands for the oddball stimulus around the same time window when P300 activity occurs suggesting possible role of cerebellum in the processing of attention-related processes. Conclusions: The results show first-in-human electrophysiological evidence of cerebellar modulation during visual attention. This confirms prior single unit recordings in monkey dentate nucleus and provides additional insight into the mechanism for non-motor functions modulated by cerebellum.

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Poster

638. Cerebellum: Non-Motor Functions

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 638.02

Topic: E.02. Cerebellum

Support: NIH 5UH3NS100543
NIH K23NS091344
NIH R61AG069729

Title: Cerebellar contributions to predictive language processing: Cerebro-dentate coherence during semantic priming in high versus low expectancy contexts

Authors: R. GOPALAKRISHNAN[1], A. SRIVASTAVA[2], O. HOGUE[3], J. BIARS[1], M. SCHROEDEL[2], K. B. BAKER[2], A. G. MACHADO[1], ***D. FLODEN**[1];
[1]Ctr. for Neurolog. Restoration, [2]Dept. of Neuroscience, Lerner Res. Inst., [3]Quantitative Hlth. Services, Lerner Res. Inst., Cleveland Clin., Cleveland, OH

Abstract: The cerebellum is thought to facilitate motor function using internal models to predict and control sensorimotor outcomes. fMRI and non-invasive stimulation studies suggest that cerebellum may use similar methods to facilitate language processing, as well. We recorded electrophysiological activity from dentate nucleus and scalp during a semantic priming task in eight patients enrolled in a clinical trial of dentate stimulation to treat post-stroke upper extremity motor impairments. Patients viewed 440 word pairs (prime word followed by a probe word) in a low-expectancy condition (LOW; 18% of pairs were related) followed by a high-expectancy condition (HIGH; 73% of pairs were related). There were also 80 word-symbol pairs (prime word followed by a group of punctuation signs) in each condition as a low-frequency (15% probability), non-semantic control. Subjects pressed a key when they saw an animal name. All animal trials were rejected to eliminate motor contamination. Scalp EEG time-locked to probe words revealed the typical N400 event-related potential at centro-parietal sites between 300-500ms reflecting semantic processing; N400 was absent to symbols. The N400 was larger to unrelated compared to related probe words in the HIGH condition, consistent with prior studies. Debiased weighted phase lag index analysis revealed significant phase coherence in the alpha band between scalp electrodes and the dentate electrode. Cluster analyses indicated two distinct regions (frontal and parietal) that showed coherence with dentate. Granger causality analysis revealed greater bidirectional signaling in the HIGH condition when predictions were violated (unrelated or symbols) relative to the LOW condition; cerebellar-to-parietal signaling was greater early in the trial (up to 400ms) while parietal-to-cerebellar signaling was greater 300-500ms corresponding to the N400 window. Greater dentate-to-frontal signaling was also observed when predictions were violated (unrelated or symbols) in the HIGH condition; this was maximal between 500-700ms suggesting a post-semantic feed-forward signal. These findings suggest that the cerebellum is selectively engaged when predictions regarding upcoming semantic content are violated and does not respond to the semantic content, per se.

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Poster

638. Cerebellum: Non-Motor Functions

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 638.03

Topic: E.02. Cerebellum

Support: NIH NINDS R01NS105899

Title: Facial emotion recognition in patients with middle cerebral artery stroke

Authors: *M. PORTER[1], O. HOGUE[2], J. BIARS[3], M. SCHROEDEL[3], K. BAKER[4], A. MACHADO[4], D. FLODEN[3];
[1]Neurosciences, [2]Quantitative Hlth. Sci., [3]Neurolog. Inst., [4]Neurosciences, Neurolog. Inst., Cleveland Clin., Cleveland, OH

Abstract: Functional MRI and behavioral studies suggest that the cerebellum is involved in facial emotion recognition (FER), the ability to identify emotional states by interpreting facial expressions. FER may be disrupted by damage to brain structures and networks associated with affective processing. Affective functions may be lateralized in cortex with right hemisphere specialization for negative affect and left hemisphere specialization for positive affect. Here, we summarize behavioral FER task outcomes to provide a framework to approach concurrent neurophysiological recordings from intact contralateral cerebellum in a small sample of patients with stroke. We hypothesized that patients will have high accuracy/short response times when identifying neutral faces. Patients with right hemisphere lesions will have low accuracy/long response times when identifying negative emotions, while patients with left hemisphere lesions will have low accuracy/long response times when identifying positive emotions. Eleven patients with chronic, focal ischemic lesions resulting from middle cerebral artery stroke (7 right hemisphere, 4 left hemisphere), completed an FER task. Patients were enrolled in a clinical trial of cerebellar deep brain stimulation to promote motor recovery after stroke and completed the FER task as part of their trial participation. Participants viewed 80 photos of 20 actors' faces expressing 4 emotions(anger, disgust, happiness, neutral). They were instructed to strike a key to indicate which emotion was depicted. Response accuracy and reaction time were recorded. Patients performed the task over 2 days, for a total 160 trials overall. Inconsistent with our hypotheses, patients with right-hemisphere strokes were less accurate when identifying neutral faces. There was no difference between left and right hemisphere patients with respect to positive emotion. One patient with a left small putamen/external capsule lesion demonstrated isolated emotion recognition deficits suggesting strategic lesions within emotional processing networks. Right-hemisphere stroke patients showed problems identifying anger. Our data support a right-hemisphere role in processing negative emotions. We did not find evidence of a left-hemisphere role in processing positive emotions. Characteristics of individual lesions may contribute to our findings. Future analyses will apply task performance to the interpretation of electrophysiological signals recorded simultaneously from the scalp and contra-lesional cerebellum in these patients to define the role of the cerebellum in affect recognition.

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Poster

638. Cerebellum: Non-Motor Functions

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Topic: E.02. Cerebellum

Support: NIH Grant R01MH129300
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BBRF Grant 19192

Title: Distinct cerebellar involved in cognitive emotional and social behavior

Authors: *O. CHAO[1], S. S. PATHAK[1], E. M. F. DE VELASCO[2], Y.-M. YANG[1,3];
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Abstract: The cerebellum processes a broad spectrum of functions, including sensorimotor as well as cognition, emotion, and social behavior, which is critically involved in neuropsychiatric disorders. The diversity of cerebellar functions is associated with its downstream connections to multiple cortical and subcortical regions through two major nuclei: the ventrolateral nucleus of thalamus (vTH) and ventral tegmental area (VTA). However, it remains unclear how the cerebellum-vTH and cerebellum-VTA circuits modulate the distinct functions. To this end, we conducted adeno-associated virus (AAV)-based anatomical tracing in mice and found that neurons in the cerebellar nuclei can project to both vTH and VTA. To selectively manipulate either the cerebellum-vTH or cerebellum-VTA circuit, we applied a Cre-LoxP guided chemogenetic approach with a dual-virus strategy. We showed that (1) sensorimotor and emotion related behaviors were disrupted by excitation, but not inhibition, of either circuit; (2) cognitive performance was impaired by excitation and inhibition of either circuit; (3) social behavior was affected by excitation and inhibition of the cerebellum-VTA, but not cerebellum-vTH, circuit. *In vivo* Ca^{2+} imaging with a genetically encoded Ca^{2+} indicator (GCaMP6f) from the vTH or VTA neurons that connected with the cerebellum further demonstrated their preferential correlation with the respective behaviors. Collectively, our findings shed light on the circuitry basis for distinct cerebellar functions and may help develop therapeutics for cerebellum-involved neuropsychiatric disorders.

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Poster

638. Cerebellum: Non-Motor Functions

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Topic: E.02. Cerebellum

Support: Ministry of Education, Culture, Sports, Science and Technology of Japan 18H05523
Ministry of Education, Culture, Sports, Science and Technology of Japan 21H04810

Title: Predictive timing signals during rhythm processing are effector-selective in the striatum but not in the cerebellum

Authors: *M. KAMEDA[1], M. TANAKA[2];
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Abstract: Both the cerebellum and striatum are involved in the perception and generation of rhythm. Previous studies have shown that neurons in the cerebellar dentate nucleus (DN, Ohmae et al., 2013, J Neurosci) and caudate nucleus (Cd, Kameda et al., 2019, eLife) exhibit periodic firing modulation in monkeys performing an omission oddball detection task that requires temporal prediction of isochronous repetitive visual stimulus. Since the magnitude of periodic activity in the Cd depends on the direction of the subsequent saccade, but not in the DN, their roles in motor preparation might be different. To clarify this, we trained three monkeys to report the stimulus omission by making saccades or hand movements.So far, we have examined 24 and 67 neurons in the Cd and DN, respectively. About half of these neurons showed significant modulation of the magnitude of periodic activity for three types of behavioral responses (button release, ipsilateral and contralateral saccades, 12/24 and 28/67, ANOVA, $p < 0.05$). Although the movement selectivity in individual neurons was stronger in the Cd than DN ($p < 10−5$), there was no specific motor preference in the population of Cd neurons (ANOVA, $p = 0.07$), and DN neurons showed a weak preference for trials requiring saccades over hand movements (Tukey, $p < 0.05$). This slight saccade bias observed in the DN may reflect differences in the cognitive demands of trials with and without saccade targets. In fact, animals made premature responses before stimulus omission more frequently in saccade trials than in hand trials (13 vs. 4%, $p < 10−17$). In the DN, periodic neuronal activity before a premature response was greater than that before a correct response ($n = 44$, $p = 0.005$). To test whether the effector selectivity exists even in the absence of temporal prediction, we also introduced the task of detecting a color oddball. The periodic activity in both brain regions decreased greatly during the task, and the saccade preference seen in the DN disappeared (ANOVA, $p = 0.58$). In individual neurons, the accuracy of decoding behavioral responses using support vector machines was significantly reduced in DN neurons (43 to 36%, omission vs. color, chance level 33%, paired t-test, $p < 10−8$) but retained in

Cd neurons (41 to 38%, p = 0.07), indicating that motor selectivity in the Cd was shown to be largely independent of temporal prediction. Taken together, these results suggest that during rhythm processing, the striatum may be involved in effector-specific periodic motor preparation, while the cerebellum may be involved in periodic sensory prediction and temporal attention.

Disclosures: M. Kameda: None. **M. Tanaka:** None.

Poster

638. Cerebellum: Non-Motor Functions

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 638.06

Topic: E.02. Cerebellum

Support: Ministry of Education, Singapore, MOE AcRF Tier 3 Award MOE2017-T3-1-002
Ministry of Education, Singapore, MOE AcRF Tier 2 Award MOE2016-T2-1-097
Interdisciplinary Graduate School bench fees

Title: Cerebellar modulation of anxiety: serotonergic involvement examined via a new 5-HT sensor

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Abstract: Cerebellar involvement in anxiety and anxiety-related behavior is supported by numerous clinical and animal studies (Behav. Brain Res. 112:107). Because the neuromodulator serotonin (5-HT) is well-known to play a role in anxiety (Nature 537:97) and serotonergic axons are amongst the most abundant fibers in the cerebellar cortex (Neuroscience 462:106), we determined whether cerebellar 5-HT is involved in anxiety. We first identified a locus for anxiety within the mouse cerebellum. We found that photostimulation of lobule VII interneurons in transgenic mice expressing channelrhodopsin in molecular layer interneurons (Cell Rep. 7:1601) caused a 67% decrease in time spent in the open quadrants of an elevated-zero maze (EZM; n = 8), indicating an anxiogenic effect. This is consistent with a previous observation that inhibiting lobule VII interneurons decreases anxiety (eLife 7: e36401) and indicates that lobule VII output is important for anxiety. To measure 5-HT levels in lobule VII, we expressed a new fluorescent 5-HT indicator ($GRAB_{5HT2h}$) with improved sensitivity to 5-HT compared to previous sensors (Nat. Neurosci. 24:746). $GRAB_{5HT2h}$ fiber photometry revealed higher 5-HT levels when mice were in open quadrants of the EZM, compared to the closed quadrants, indicating that 5-HT is higher during a low-anxiety state. To determine whether 5-HT levels in lobule VII affect anxiety, we optogenetically controlled the activity of serotonergic axons in lobule VII of a transgenic

mouse line expressing channelrhodopsin or archaerhodopsin in serotonergic neurons (PNAS 102:16472). Photostimulation of 5-HT fibers caused a 106% increase in the amount of time spent in open quadrants of the EZM (n = 8), indicating an anxiolytic effect. Conversely, photoinhibition of 5-HT fibers produced an 81% decrease in the time spent in the open quadrants (n = 4), indicating an anxiogenic effect. Collectively, these results support the hypothesis that 5-HT levels in cerebellar lobule VII regulate anxiety, with higher 5-HT reducing anxiety.

Disclosures: P.W. Chin: None. **J. Wan:** None. **Y. Li:** None. **G.J. Augustine:** None.

Poster

638. Cerebellum: Non-Motor Functions

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 638.07

Topic: E.02. Cerebellum

Support: NIH Grant RO1 NS106915
VA Grant IO1 BX003893

Title: The role of the cerebellum in the extinction of associative fear memory

Authors: *R. BHUVANASUNDARAM, M. A. FAROOQ, S. LIU;
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Abstract: The cerebellum, classically known for its role in motor control, has recently also been implicated in non-motor functions. Fear learning and memory and the extinction of fear memory contribute to various psychological disorders including PTSD. Fear memory extinction training has been used as a therapeutic tool for patients suffering from fear-related psychological disorders. Although the cerebellum is involved in associative fear learning and memory, whether the cerebellum contributes to the extinction of fear memory is not known. We tested this idea in mice using chemogenetics combined with fear conditioning and extinction behavioral paradigms. We first addressed whether inhibiting Purkinje cell (PC) activity would affect extinction learning and memory. Animals underwent fear conditioning training with 8 tone and shock pairings and one extinction session with 20 tones in a new context 24 hours later. This was then followed by an extinction memory retention test (“recall”) the next day. In the first experiment, we injected L7-Cre/Gi-DREADD male and female mice with clozapine N-oxide (CNO) to activate Gi-DREADD 30 min before extinction training. The freezing response to tone during recall was significantly higher in L7-Cre/Gi-DREADD male mice than in the saline-injected control group. Administration of CNO to L7-cre mice did not impair extinction memory. By contrast, the freezing percentage did not significantly change in L7-Cre/Gi-DREADD female animals administered with CNO. Next, we tested whether PC activity during extinction learning or consolidation is required for extinction memory. We injected L7-Cre/Gi-DREADD mice with CNO immediately after the mice had undergone extinction training. Inhibition of PC activity during the consolidation period did not affect the tone-induced freezing response during recall in

either male or female mice, suggesting PC activity during extinction training is necessary for extinction memory formation. We then tested whether inhibition of PC activity without extinction training affected memory retention. L7-Cre/Gi-DREADD mice received CNO or saline injection one day after fear conditioning without undergoing extinction training. Suppression of PC activity did not alter the freezing response during recall the next day. Our results show that cerebellar PC activity during training, but not during consolidation, is critical for subsequent extinction memory formation in male mice.

Disclosures: R. Bhuvanasundaram: None. **M.A. Farooq:** None. **S. Liu:** None.

Poster

638. Cerebellum: Non-Motor Functions

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 638.08

Topic: G.09. Drugs of Abuse and Addiction

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Ministerio de Ciencia e Innovación Grant PGC2018-095980-BI00/ MCI/AEI/FEDER, UE
Fulbright Fellowship Grant PRX18/00012

Title: Activity and plasticity regulation in the Infralimbic-Cerebellum pathways in cocaine-induced preference memory

Authors: *J. GUARQUE-CHABRERA[1,2], K. KHODAKHAH[1], M. MIQUEL[1,2];
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Abstract: Reciprocal pathways connecting the cerebellum and the medial prefrontal cortex (mPFC) provide a biological and functional substrate of their codependence to modulate cognitive and motivational functions. Dysfunction of both areas underlie the phenotypes of several neuropsychiatric disorders, and may operate as a risk factor for drug addiction. Miquel's lab has reported that both infralimbic cortex (IL) and cerebellum exhibit hallmark signatures of drug-induced memories, such as cFos and perineuronal nets (PNNs). Moreover, either a lesion in the vermal lobule 8 (L8) of the cerebellum or an IL deactivation facilitated the preference for cocaine-related cues, however, simultaneous L8-IL deactivations abolished this effect. Therefore, cerebellum-IL functional relationships might be compensatory. One of the relays for the cerebellum to modulate mPFC functions is the VTA. Indeed, Khodakhah's lab was able to modulate reward-related behaviors by using optogenetic manipulation of the cerebellar outputs targeting the VTA.
Here we sought to further investigate the cerebellum-infralimbic hallmark signatures of drug-induced memories and the anatomical pathways whereby both structures reciprocally might

modulate each other. To do so we 1) inactivated IL and assessed activity and PNN expression in the posterior cerebellum, 2) digested PNNs in L8 at different time points of the learning process using the enzyme *chondroitinase ABC*, and 3) using different tracing agents we created an anatomical map of the ascending and descending cerebellum-IL reciprocal pathways.
First, IL inactivation encouraged the acquisition of cocaine-induced memory and increased cFos, vGluT2 activity around neurogranin+ Golgi interneurons, and PNN expression in the posterior cerebellum. Second, the enzymatic digestion of PNNs in L8 disrupted short-term cocaine memory and facilitated reinstatement of the original cocaine-memory by preventing the consolidation of extinction. Third, our anatomical maps show that L8 through the interposed DCN might contact VTA and then contact IL, and IL might send projections to both interposed DCN and inferior olive and these areas then will reach L8.

Disclosures: J. Guarque-Chabrera: None. **K. Khodakhah:** None. **M. Miquel:** None.

Poster

638. Cerebellum: Non-Motor Functions

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Topic: G.09. Drugs of Abuse and Addiction

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NIDA Grant R1DA044761
NIMH Grant R01MH115604

Title: Cerebellar dysfunction in animal models of neurodevelopmental disorders with human mutations in the CACNG2 gene

Authors: *N. BELTRÃO[1,2], G. L. CALDEIRA[2], Â. S. INÁCIO[2], R. MACEDO[2,3], M. V. RODRIGUES[2], O. ANTAL[2], K. KHODAKHAH[1], A. L. CARVALHO[2,3];
[1]Dominick P. Purpura Dept. of Neuroscience, Albert Einstein Col. of Med., Bronx, NY; [2]Ctr. for Neurosci. and Cell Biol., Coimbra, Portugal; [3]Dept. of Life Sci., Univ. of Coimbra, Coimbra, Portugal

Abstract: The cerebellum, a brain structure best known for its role in motor coordination, has recently gained center stage in the field of neuropsychiatric disorders. In fact, it is now known that cerebellar dysfunction can lead to social and cognitive deficits observed in Autism Spectrum Disorder, Intellectual Disability and Schizophrenia patients. Despite this recent evidence, the disease mechanisms linking cerebellar dysfunction to neuropsychiatric disorders are still unclear. In this work we are studying cerebellar dysfunction in two knock-in (KI) mouse models expressing intellectual disability (STG-VL) and schizophrenia-associated (STG-SN) human

mutations in the *CACNG2* gene. This gene encodes for the synaptic protein stargazin (STG) that is implicated in the synaptic targeting of AMPA receptors (AMPAR).
Our results have revealed that mice expressing STG-VL show impaired motor learning and display a decreased preference for social novelty. Moreover, analysis of cerebellar slices suggests that these KI animals have a decreased cerebellar volume and have shorter and less complex Purkinje cells. We have also found that cerebellar postsynaptic densities (PSD) isolated from these animals have decreased STG and GluA2-AMPAR subunit levels. Interestingly, while STG-SN expressing mice show impaired motor learning, only female STG-SN KI animals display decreased preference for social behavior and social novelty. Analysis of cerebellar PSDs from these animals also suggests alterations in synapse composition.
Taken together, these results unveil a new role for STG in cerebellar function, indicate that different mutations in the STG-encoding gene lead to shared and distinct defects, and suggest that synaptic dysfunction in this region may underlie the development of neuropsychiatric disorder-associated phenotypes.

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Poster

638. Cerebellum: Non-Motor Functions

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 638.10

Topic: G.09. Drugs of Abuse and Addiction

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Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital Pre-doctoral fellowship ACIF/2019/109
Universitat Jaume I Pre-doctoral fellowship PREDOC/2019/35

Title: Competition between learning and drugs of abuse for cerebellar resources

Authors: *P. IBÁÑEZ-MARÍN, A. SANCHEZ-HERNANDEZ, E. MARÍN-SAMPIETRO, O. RODRÍGUEZ-BORILLO, L. ROSELLÓ-JIMÉNEZ, L. FONT, M. MIQUEL;
Àrea de Psicobiologia, Departament de Psicologia Bàsica, Clínica i Psicobiologia, Univ. Jaume I, Castelló de la Plana, Spain

Abstract: Abstract
Previous research has demonstrated that prolonged experience with drugs can lead to an imbalance between flexibility and automatic repetition that bias action selection towards insensitive to reward devaluation. Recent studies by our group have shown that cocaine-induced

incentive memory and extended access to cocaine self-administration increased neural activity and the expression of specialized extracellular matrix structures that stabilize synapsis called perineuronal nets (PNNs) in the cerebellum. In the present study, we aimed to investigate the cerebellar correlates of insensitivity to devaluation in naturally rewarded behaviors after long-lasting drug experience. Prior or after extended treatment in ascendent doses with cocaine (2mg/kg, 4mg/kg and 10 mg/kg for 7 weeks) or alcohol concentrations (2,5%, 5% and 10% for 12 weeks), rats were trained in operant boxes to consume two kinds of palatable pellets (banana and chocolate) in a random ratio schedule. Then, the reward was devalued by satiety (1h of pre-feeding) and sensitivity to reward devaluation was assessed under extinction in two tests of 5 min. *c-Fos* and PNN expression in the cerebellar cortex and deep nuclei were assessed. Results showed that drug-treated rats showed sensitivity to reward devaluation like the control group although the proportion of animals that exhibit insensitivity is higher in drug-treated rats. In addittion, we found that extended drug treatment decreased the number of *c-Fos*+ Purkinje cells and the intensity and integrity of PNNs, only when the drug was administered before any learning. Drug treatment after prior instrumental learning increased *c-Fos*+ Purkinje cells. Our findings indicate that a previous experience can modulate the effects of drugs of abuse on the cerebellum and regulate cerebellar output.

Disclosures: P. Ibáñez-Marín: None. **A. Sanchez-Hernandez:** None. **E. Marín-Sampietro:** None. **O. Rodríguez-Borillo:** None. **L. Roselló-Jiménez:** None. **L. Font:** None. **M. Miquel:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.01

Topic: E.02. Cerebellum

Support: ERC CN Identity 852869
NWO VIDI 192008

Title: A direct cerebral cortical-cerebellar nuclei pathway and its roles in movement control

Authors: *C. B. SCHAEFER[1], A. LI[2], Y. ADOLFS[3], B. L. BOUWEN[4], H. HASANBEGOVIC[5], R. J. PASTERKAMP[3], C. DE ZEEUW[5], X. LI[6], H. GONG[6], F. E. HOEBEEK[7], Z. GAO[5];
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Abstract: The execution of volitional movements is mediated by neuronal communication across inter-connected brain areas, including motor cortex, cerebellum and brainstem. It is generally acknowledged that motor cortex exerts its control over cerebellum mainly via the cortico-pontine-cerebellar pathway. Here we describe a novel circuit for direct cortico-cerebellar communication. We found that a subgroup of layer 5 pyramidal neurons directly projects to cerebellar nuclei (CN), the output region of cerebellum, which we termed as cerebral cortico-cerebellar nuclei (CC-CN) projecting neurons. Using retrograde viral tracing we localized the distribution of CC-CN projecting neurons in motor cortex, prefrontal cortex and insular cortex. Focusing on the motor cortical regions, rabies virus assited trans-neuronal tracing reveal that the CC-CN projecting neurons receive pronounced inputs from local cortical networks. Anterior grade tracing and single axon reconstruction using fMOST technique highlighted a wide spread projection patterns of this group of neurons. Optogenetic stimulation of the axons of CC-CN projecting neurons drives CN activity in awake mice. Furthermore, we evaluated the behavioural significance of the CC-CN connection using a goal directed forelimb reaching paradigm. Two photon Ca^{2+} imaging identified task-related modulation patterns of CC-CN projecting neurons. Optogenetic perturbation of the CC-CN pathway disturbs movement execution. These results provide for the first time an integrated anatomical, physiological and functional insights into the roles of CC-CN pathway. Our study indicate that a discrete group of motor cortical neurons could integrate local cortical signals and transfer an efference copy directly to the cerebellar output regions. This finding broadens the palette of circuits mechanisms for cortico-cerebellar communication and suggests that motor cortex may directly influence the ipsilateral cerebellar output in movement control.

Disclosures: C.B. Schaefer: None. **A. Li:** None. **Y. Adolfs:** None. **B.L. Bouwen:** None. **H. Hasanbegovic:** None. **R.J. Pasterkamp:** None. **C. De Zeeuw:** None. **X. Li:** None. **H. Gong:** None. **F.E. Hoebeek:** None. **Z. Gao:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.02

Topic: E.02. Cerebellum

Support: FNRS and UMons Fund

Title: Electrical stimulation of the somatosensory cortex evoked the recruitment of the olivocerebellar module in awake mice.

Authors: G. CHERON[1], A. CABALLERO-TAPIA[2], D. RISTORI[1], J. MÁRQUEZ-RUIZ[3], A.-M. CEBOLLA[1], L. RIS[2];
[1]LNMB, Univ. Libre de Bruxelles, Brussels, Belgium; [2]Dept. of Neurosciences, Mons Univ., Mons, Belgium; [3]Dept. of Physiology, Anat. and Cell. Biol., Pablo de Olavide Univ., Seville, Spain

Abstract: Recent evidence demonstrate the key role of the cerebellum in motor and nonmotor domains through a great number of cerebro-cerebellar closed loops that sustain different forms of neural plasticity. In this context, the long-range bidirectional connections between the cerebral cortex and the cerebellum are assumed by the most prominent fiber bundles (the cerebral and the medial cerebellar peduncles) in the mammalian brain[1]. The communication timing between these two important entities is crucial for a better understanding of the related functions. We here explored the firing activities of the neurons in the deep cerebellar nuclei (DCN) (interpositus nucleus) and the Purkinje cells (Crus I -II) during the electrical stimulation of the contralateral primary somatosensory cortex (S1) (whiskers related area) in the awake mice (n=8). We showed that the electrical stimulation (single pulse of 0.2 ms given at a random rate 0.5 to 1 Hz) of S1 evoked: (1) an increase of the firing rate of the DCN neurons (from 47.3 ± 36.3 Hz before to 82.4 ± 61.1 Hz after the stimulus) at a mean onset latency of 5.9 ± 3.7 ms (n=30); (2) an increase of the simple spike (SS) of the Purkinje cell (PC) (from 100 ± 37.9 Hz before to 341 ± 145 Hz) at a mean onset latency of 9.6 ± 3.0 ms (n=20) and (3) the occurrence of a complex spike (CS) at the mean latency of 17.1 ± 2.9 ms (n=20). For about 25% of the DCN neurons, the initial increase of the firing rate was interrupted by a firing decrease occurring at a mean latency of 19.6 ± 8.3 ms. In addition, we also found that S1 stimulation strongly excites the Golgi cells at about 5 ms of latency. This timing organization is compatible with the activation times obtained by whisker pad stimulation. The recruitment by S1 of the 3 key neuronal units (DCN, CrusI-II PC and inferior olive) of the olivocerebellar module corroborates the idea that the somatosensory cortex provides an important input to the cerebellar module which can be treated and compared to the more direct peripheral input. We discuss the relevance of these relative timing in favor of a tactile feedback send by S1 to update the internal forward models of the cerebellum[2].
References: 1.Suzuki, L., Coulon, P., Sabel-Goedknegt, E.H., & Ruigrok, T.J.H. (2012) *J. Neurosci. Off. J. Soc. Neurosci.*, **32**, 10854-10869.2. Kilteni, K. & Ehrsson, H.H. (2020) *J. Neurosci. Off. J. Soc. Neurosci.*, **40**, 894-906.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.03

Topic: E.02. Cerebellum

Support: CIHR Grant FDN-143209

Title: Functional Mapping of Mouse Cortico-Cerebellar Connectivity During Distinct Behavioral States

Authors: *Y. YAN[1], T. MURPHY[2];
[1]Univ. of British Colombia, Univ. of British Colombia, Vancouver, BC, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Accumulating evidence from human and animal studies have shown important roles the cerebellum plays in extraordinarily diverse functions such as motor learning, sensory processing, reward, feeding and social behavior, but where and how the cerebellum receives these diverse streams of information from the rest of the brain remains unclear. Here, we study functional connections between the cerebellum and the dorsal cortex of the mouse brain. We used Neuropixels probe and mesoscale Ca2+ imaging to record spiking activity of individual cerebellar neurons and population activity of the entire dorsal cortex. We then constructed cortical functional connectivity patterns of individual cerebellar neurons by correlating cerebellar activity with cortical activity. We found that ~1/3 (1906/5382) of the recorded cerebellar neurons show stable connectivity patterns with the dorsal cortex. These neurons exhibit broad profiles of cortical representations, and these representations are anatomically defined, consistent with intrinsic cortical activity motifs and can dynamically change between anesthetized and awake states. Interestingly, the cerebellar neurons that exhibit similar cortical connectivity patterns are not topographically organized within the cerebellum itself; instead of physical distance, the local synaptic coupling between a pair of cerebellar neurons is a better indicator of whether they are affiliated with similar long-range functional networks. Furthermore, we showed that the simple spikes and complex spikes of cerebellar Purkinje cells can exhibit distinct or linked cortical mesoscale representations, indicating their unique capabilities in integrating information of multiple modalities. Lastly, we showed that these spontaneous connectivity patterns generated using resting state activity can predict cerebellar neurons' functional responses to external sensory stimuli. Together, our results provide extensive description on the state-dependent landscape of cortical-cerebellar functional connectivity which adds to our current understanding of how the cerebellum fits into the inter-regional network. Our data also provides strong evidence that behavioral-related information of individual neurons can be decoded from their resting state activity patterns.

Disclosures: Y. Yan: None. **T. Murphy:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.04

Topic: H.09. Spatial Navigation

Support: NIH Grant 5R01MH112143-05

Title: Cerebellar interactions with the cerebral cortex in healthy and ataxic mice

Authors: *B. L. CORREIA[1], M. DHAMALA[2], R. SILLITOE[3], Y. LIU[1], D. H. HECK[1]; [1]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]Georgia State Univ., Atlanta, GA; [3]Baylor Col. of Med., Houston, TX

Abstract: Long known for its role in motor control, it is increasingly clear that the cerebellum is also involved in numerous cognitive and affective behaviors. Though the neuronal mechanism for the role of the cerebellum in cognition is still unclear, there is a consensus that it involves cerebellar interactions with the cerebral cortex. Recent studies suggest that the cerebellum monitors, and possibly coordinates, the precise phase alignment or coherence of neuronal oscillations in cerebral cortical areas. Specifically, we previously showed that Purkinje cells in right lobulus simplex (LS) and Crus I of healthy mice represent the instantaneous phase and phase differences of local field potential (LFP) oscillations in the medial prefrontal cortex (mPFC) and the dorsal hippocampal CA1 (dCA1). Additionally, we have shown that optogenetic stimulation of right LS impairs spatial working memory performance and mPFC-dCA1 coherence modulation. Here, we asked how loss of cerebellar function affects the interactions of LFP oscillations between the cerebellum, mPFC, and dCA1 using multisite *in vivo* extracellular recordings in freely moving mice. We compared a mouse model of cerebellar ataxia and their littermate controls. The ataxic mice were designed to have a genetically induced loss of Purkinje cell neurotransmission, resulting in an expected repertoire of cerebellar motor deficits. Here we asked whether these mice also have neurophysiological defects identifiable in the neuronal oscillations of LFP signals that could be indicative of cognitive circuit dysfunction. We quantified power spectra of LFP oscillations in each structure, the magnitudes of coherence of oscillations, and Granger causality (GC) between each pair of structures using a nonparametric spectral method. Resting-state coherence of gamma oscillations between cerebellar LS and mPFC was significantly increased in ataxic mice relative to healthy controls. Similarly, GC analysis revealed significantly larger GC from the mPFC to cerebellar LS in the gamma frequency range in ataxic compared to healthy control mice. Ataxic animals also exhibited larger maximal dCA1-mPFC gamma coherence values. Our findings reveal that Purkinje cell neurotransmission is required for normal functional interactions between the cerebellum and cerebral cortex and between cerebral cortical areas involved in cognitive functions, further supporting an involvement of the cerebellum in the modulation or coordination of functional communication between brain areas.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

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Program #/Poster #: 639.05

Topic: E.02. Cerebellum

Support: Institute for Social Science Research (ASU Internal Funding)
Arizona Alzheimer's Consortium

Title: Modulation of cognitive behavior by the deep cerebellar nuclei in juvenile life

Authors: *T. LYLE, A. VORA, J. ESPINOZA, J. L. VERPEUT;
Psychology Dept., Arizona State Univ., Tempe, AZ

Abstract: Atypical cerebellar and neocortical development are hallmarks of autism spectrum disorder (ASD). However, it is unknown how long-distance connectivity between the cerebellum and neocortex influence neural development and behavior. The cerebellum has multisynaptic connections through the deep cerebellar nuclei (DCN) and thalamus to neocortical cognitive regions, yet the role of the DCN during critical periods remains largely unknown. Moreover, the function of DCN perineuronal nets (PNNs), specialized extracellular matrix structures whose appearance is associated with the end of the critical period of plasticity, are undefined. We hypothesized that typical maturation of the cerebello-neocortical circuit is required to relay input into distal neocortical regions for cognitive behavior. To examine cerebellar modulation of cognitive behavior in juvenile mice, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs; AAV2-hSyn-hM3D(Gq)-mCherry) were used to enhance DCN activity in both male and female mice via the ligand, clozapine-N-oxide (CNO; 10mg/kg), in drinking water from postnatal day 21-35. Cognition was analyzed using a touch screen device to examine visual discrimination and reversal performance. DCN activation improved reversal performance ($p<0.05$) only in females, but did not alter learning the task or visual discrimination performance. Interestingly, females completed more trials ($p<0.01$), as well as, initiated ($p<0.001$) and responded to trials faster ($p<0.05$). We analyzed DCN PNNs using Wisteria Floribunda Lectin (WFA). Female controls had higher WFA intensity than male controls ($p<0.001$). DCN activation reduced WFA intensity ($p<0.001$, Cohen's $d = 3.8$) in females. We found a negative correlation between WFA intensity and reversal performance ($R = -0.59$, $p<0.001$), suggesting that reducing PNNs improves cognitive flexibility. Ongoing studies are exploring DCN activation on PNNs in males and changes in neural structure in neocortical regions receiving DCN input.Overall, juvenile DCN activation increased reversal performance and reduced WFA intensity. These studies will establish a role for the DCN in modulating cognition during neurodevelopmental critical periods of plasticity.

Disclosures: T. Lyle: None. **A. Vora:** None. **J. Espinoza:** None. **J.L. Verpeut:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.06

Topic: E.02. Cerebellum

Support: K99NS110978
F32NS101889
R35NS097284
R01NS032405

Title: A Purkinje cell to parabrachial nucleus pathway enables broad cerebellar influence over the forebrain and emotional valence

Authors: *C. CHEN[1], L. N. NEWMAN[2], A. STARK[3], W. REGEHR[1];
[1]Harvard Med. Sch., Harvard Med. Sch., Boston, MA; [2]Harvard Univ., Harvard Univ., Hull, MA; [3]Northeastern Univ., RIVERHEAD, NY

Abstract: In addition to its well-known contributions to motor control and motor learning, the cerebellum is involved in language, emotional regulation, anxiety, and affect1-4. We found that suppressing the firing of cerebellar Purkinje cells (PCs) rapidly excites forebrain areas that could contribute to such functions, including the amygdala, basal forebrain, and septum, but that the classic cerebellar outputs, the deep cerebellar nuclei (DCN), do not project to these regions. Here we show that parabrachial nuclei (PBN) neurons that receive direct PC input, project to and influence all of these forebrain regions and many others. Furthermore, the function of this pathway is distinct from the canonical pathway: suppressing PC to PBN activity is aversive, whereas suppressing the PC to DCN pathway is rewarding. Therefore, the PBN pathway allows the cerebellum to influence the entire spectrum of valence, modulate the activity of forebrain regions known to regulate diverse nonmotor behaviors, and may be the substrate for many nonmotor disorders related to cerebellar dysfunction.

Disclosures: C. Chen: None. **L.N. Newman:** None. **A. Stark:** None. **W. Regehr:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.07

Topic: E.02. Cerebellum

Support: R01 Grant MH115604

Title: A potential role for a direct Cerebellar-Hypothalamic pathway in Social Behavior.

Authors: *N. S. CAYLA, M. OÑATE, I. AZINGE, L. SPAETH, J. VERA, K. KHODAKHAH; Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The cerebellum is well-known for its involvement in motor control and coordination, but recent clinical and scientific observations have led to a growing interest in its roles in the regulation of cognitive and emotional processes. For instance, there is some evidence in the literature for the presence of cerebellar projections to the hypothalamus, one of the emotional

control center. Taking advantage of viral anatomical tracing techniques, we explored whether there are direct projections from the main output of the cerebellum, the Deep Cerebellar Nuclei (DCN), to the hypothalamus. Further, we explored the identity of neurons in the hypothalamus that receive direct inputs from the DCN. We found that the DCN directly targets neurons in several hypothalamic regions, including the Paraventricular Nucleus of the Hypothalamus (PVN), which controls myriad behaviors such as mating, parenting, aggression, appetite, and stress. Labeling with selective antibodies revealed that some of these cells were oxytocin- and arginine vasopressin-expressing neurons, two neuropeptides known as important modulators of diverse social behaviors. To explore whether these cerebellar projections were capable of modulating the activity of the targeted neurons, we optogenetically activated the DCN axons in the hypothalamus and examined changes in neuronal activity in the PVN. Additionally, in a three-chamber test of social behavior, we found that activation of cerebellar axons in the PVN enhanced social interaction. Taken together, our data suggest that cerebellar inputs to the PVN may contribute to the regulation of social behavior. Given that oxytocin and vasopressin also regulate other processes such as hunger and thirst, it is possible that the cerebellar projections to the hypothalamus may also contribute to other behaviors.

Disclosures: **N.S. Cayla:** None. **M. Oñate:** None. **I. Azinge:** None. **L. Spaeth:** None. **J. Vera:** None. **K. Khodakhah:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIMH Grant 5R01MH115604-06
NIDA Grant 5R01DA044761-06

Title: Neuroanatomical characterization of cerebellar inputs to the dopamine centers in the midbrain

Authors: *M. OÑATE, J. GUARQUE-CHABRERA, J. VERA, V. LOVALLO, L. KHATAMI, G. VERA, K. KHODAKHAH;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The emerging view of the cerebellum relates its function not only to motor control, but also to cognition. In agreement with this, cerebellar dysfunction is implicated in a wide variety of pathologies that involve impairments in the reward system, including addictive behavior and mental disorders like schizophrenia, and autism spectrum disorder. Previous work of our laboratory have shown that the cerebellum sends monosynaptic excitatory projections to the key regions of the midbrain dopamine system; the substantia nigra pars compacta (SNc), and the ventral tegmental area (VTA). Behavioral experiments in mice suggest that cerebellar inputs

to the VTA are rewarding and are required for normal performance in a social behavior task an lately, that inputs to SNc conveys information related to movement initiation, vigor and reward processing. Cerebellum drives the activity of SNc and VTA neurons, producing dopamine release in the dorsal striatum, prefrontal cortex and Nucleus Accumbens, the major outputs of SNc and VTA, respectively; suggesting that the cerebellum might play a role in motor and cognitive functions through dopamine modulation. Despite the physiological characterization of these cerebellar pathways, detailed characterization of the distribution and heterogeneity of the neuronal sub-populations involved is still unknown. Characterization of the neuroanatomy and connectivity of the sub-circuits is key to understand the circuit function. In this work, we performed different experiments using intersectional tracing approaches that aimed at anatomically delineating these pathways. Additionally, functional connectivity experiments add new comparative data shedding light on the detailed circuits that underlie cerebellar contribution to dopamine release.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant MH115604
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Title: The cerebellum drives fast excitation and dopamine release in the nucleus accumbens via defined projections to the VTA

Authors: *J. VERA, M. OÑATE, K. KHODAKHAH;
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Abstract: It is now clear that the cerebellum sends direct projections to the ventral tegmental area (VTA), and that the activity conveyed by these projections contributes to reward processing and social behavior. The main target of VTA dopamine neurons is the nucleus accumbens (NAc), where dopamine release is necessary to promote adaptive goal-directed behavior. The VTA and NAc generate reciprocal parallel circuits with different roles in behavior, highlighting the complexity of the mesolimbic dopaminergic system. Whether cerebellar projections to VTA shows selectivity for any of these parallel circuits remains to be investigated. Here we delineate the functional connectivity and anatomy of these disynaptic circuits.Using electrophysiology in vivo we found that optogenetic activation of cerebellar inputs to VTA drives fast activation of NAc single units with latency as short as ~4 ms. Using a fluorescent sensor and fiber photometry in vivo, we find that activation of cerebellar inputs to VTA produces a fast, strong, and

consistent increase in dopamine signals in NAc core and shell, suggesting a widespread cerebellar connection with ample regions of NAc. Anatomical characterization of the circuit reveals that VTA neurons contacted by the cerebellum densely project to the whole NAc, and that VTA neurons that convey cerebellar inputs to NAc are located in higher numbers in specific VTA subregions. Finally, using transsynaptic anatomical tracing we find that cerebellar projections to NAc shell lateral and medial, via the VTA, are composed of a non-overlapping cell population present in the three cerebellar nuclei. Taking together, our data show that the cerebellum has a robust functional and anatomical connection with the nucleus accumbens, delineating a fundamental circuit necessary to understand the role of the cerebellum in reward processing.

Disclosures: **J. Vera:** None. **M. Oñate:** None. **K. Khodakhah:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

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Program #/Poster #: 639.10

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AD was supported by NIH Grant T32 GM 007377

Title: Bridging the gap between the cerebellum and the nucleus accumbens through disynaptic functional and anatomical circuit mapping

Authors: A. F. D'AMBRA, K. VLASOV, S. JUNG, S. GANESAN, E. G. ANTZOULATOS, ***D. FIORAVANTE**;
Univ. of California Davis, Davis, CA

Abstract: The cerebellum (CB) is a key modulator of non-motor functionality including reward learning, emotional processing and social cognition. Recent studies have demonstrated CB connectivity to cortical brain regions involved in these high-order functions; however, there is still much left unknown with regards to subcortical structures. We sought to elucidate the functional and anatomical connectivity of the CB with the nucleus accumbens (NAc), a subcortical hub for social and reward behaviors. Using in vivo electrophysiological recordings in anesthetized mice, we discovered that stimulation of the CB output nuclei led to significant modulation of NAc activity, evidenced in both single- and multi-unit recordings. CB influence on NAc varied by NAc subregion, with differences emerging between NAc core and NAc shell

responses. Using anatomical tracing techniques, we identified three regions—the ventral tegmental area, the centromedial nucleus of the thalamus, and the parafascicular nucleus of the thalamus—that could mediate these NAc responses. By manipulating cerebellar inputs to each of these regions using targeted optogenetics, we confirmed that each region is a functional node serving CB-NAc connectivity. Further analysis indicated that response prevalence in NAc core vs NAc shell varied depending on which CB nodal input was stimulated. These data confer novel understanding on CB connectivity with NAc; can inform the role of the CB in reward processing and social behavior; and can offer testable mechanistic models about the contribution of CB dysfunction to substance use disorders and neurodevelopmental disorders.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

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Topic: E.02. Cerebellum

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UC Davis Dean's Distinguished Graduate Fellowship

Title: Novel cerebellar projections to limbic thalamus regulate acquired salience during extinction learning

Authors: *K. VLASOV[1], S. JUNG[1], A. F. D'AMBRA[1], J. VILLALOBOS[1], H. BARRERA[1], E. G. ANTZOULATOS[1,2], D. FIORAVANTE[1,2];
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Abstract: The cerebellum (CB) has been widely recognized as a control center for motor learning and coordination, often at the expense of its equally important role in executive and limbic functions supported by long standing evidence from human patients. The brain regions underlying cerebellar modulation of emotion have been hinted at in early electrophysiological experiments, but the pathways and mechanisms involved in this type of processing remain poorly understood. To address this knowledge gap, we undertook a viral tracing study, which uncovered a disynaptic pathway between the cerebellar output nuclei and the basolateral amygdala through the intralaminar thalamus (centromedial and parafasicular nuclei, CM and PF). Using optophysiology, we confirmed the functionality of this pathway in vivo and ex vivo. Building off

of this published work, we are currently examining recruitment dynamics of this new pathway during affective processing and the type of information it might convey to influence affective behavior. We find that optogenetic inhibition of CB-PF projections significantly enhances extinction of learned fear while inhibition during other time periods in the task, or during fear learning, has no effect. Inhibition of CB-PF signals has no effect on anxiety-related emotional state which was assessed in an elevated plus maze task, or motor control as assessed in the open field test, suggesting functional specialization. Our interpretation of these findings is that the cerebellum processes signals about acquired stimulus salience, which it communicates to limbic brain centers including the basolateral amygdala to modulate processing of learned fear. Our study provides the first evidence of a causal role for this novel cerebellar pathway in fear extinction learning. Overall, these findings advance our understanding for how the cerebellum can modulate complex cognitive and limbic functions and aim to guide future studies of how these circuits may be impaired in neuropsychiatric disorders.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

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Topic: E.02. Cerebellum

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Title: Organizational features of the locus coeruleus-norepinephrine projection to motor regions of the rodent brain

Authors: *H. PREDALE[1], N. W. PLUMMER[2], D. P. FOX[1], P. JENSEN[2], B. D. WATERHOUSE[1];
[1]Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ; [2]Neurobio., Natl. Inst. of Envrn. Hlth. Sci., Durham, NC

Abstract: The brainstem nucleus locus coeruleus (LC) projects broadly throughout the forebrain, brainstem, cerebellum, and spinal cord and is a major source for release of the small molecule transmitter norepinephrine (NE) in these areas. While we know much about the organization of the LC-NE system with respect to sensory and cognitive circuitries and the impact of LC output on sensory guided behaviors and executive function, much less is known about the role of the LC-NE pathway in motor network operations and movement control. As a starting point for closing this gap in understanding, we are using the intersectional recombinase-based viral-genetic strategy TrAC (Tracing Axon Collaterals) to characterize LC-NE projections to motor

control centers of the mouse brain. This technique allows us to not only map the distribution of LC-NE axons to target regions of the cerebellum, but also to examine the distribution of their cell bodies within the LC and their dendritic fields within the peri-coerulear space. Initial results indicate that the distribution of cells that send axons to target regions of the cerebellum, i.e. the deep cerebellar nuclei and cerebellar cortex, is significantly denser than the distribution of cells that send axons to motor thalamus. The deep cerebellar nuclei receive input from a large number of bilaterally distributed LC neurons. Lobules in the anterior cerebellum and posterior cerebellum also receive input from bilaterally distributed LC cells, but these cells are less numerous and more scattered than those projecting to the deep cerebellar nuclei. These results contrast with the scattered and predominantly ipsilateral (95%) distribution of the NE-containing cells that project from LC to cerebral cortex including the primary motor cortex. Ongoing studies are targeting the red nucleus and lateral vestibular nucleus as additional components of the central motor control network and involve quantification of the density of dendritic fields and axon collaterals stemming from LC-motor projection neurons. The clearly demarcated dendritic fields of motor-projecting LC neurons in TrAC animals allow us to consider whether these cells are afferently regulated by inputs to specific sub-regions of the peri-coerulear space. The overarching goal of this work is to determine the distribution of motor projection neurons within LC, visualize their dendritic fields within the peri-coerulear space, and characterize the distribution of their axon collaterals. The expectation is that this information will establish a foundation for future studies aimed at elucidating the role of the LC-NE system in motor control.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

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Topic: E.02. Cerebellum

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Title: Noradrenergic tone modulates cerebellar nuclear activity-dependent performance in delay eyeblink conditioning

Authors: *J. LEE, H.-J. BOELE, W. T. FLEMING, G. J. BROUSSARD, F. D'OLEIRE UQUILLAS, S. S.-H. WANG;
Princeton neuroscience institute, Princeton Univ., Princeton, NJ

Abstract: Noradrenergic tone regulates learning and performance in forebrain structures, but little is known about how this pathway affects cerebellar function. We administered a delayed eyeblink conditioning task to mice, and simultaneously recorded activity from populations of locus coeruleus (LC) axons and deep-nuclear neurons in the interpositus nucleus (InT) using fiber photometry. We used a genetic strategy to express GCaMP8f in LC axons and jRGECO1a in local neurons of the InT to allow multiplex imaging of both target cell types in InT. A 280 ms blue LED light served as the conditioned stimulus (CS), and a co-terminating corneal airpuff (30 ms) as the unconditioned stimulus (US). Conditioned responses (CRs) developed over the course of 18 daily sessions, with 90-100 trials per session. CS-evoked InT signals grew on a session-by-session basis (linear mixed-effects model, LME, effect size: Cohen's d = 1.16, p = 1.0e-06) and with learning stage (sessions with less than 20 CR% were classified as before learning, 20 to 60% as during learning, and more than 60% as after learning; linear regression by stage, effect size: Cohen's d = 0.59, p = 0.06). However, on a trial-by-trial basis, InT signals only weakly predicted CR amplitude (LME for trial-by-trial: d = 0.18, p = 3.8e-15). To test whether pre-CS noradrenaline levels also couple InT activity and CRs, we quantified the ability of pre-CS LC activity ("LC tone") to moderate the trial-by-trial relationship between CS-evoked InT and CR amplitude. LC tone was Z-scored and defined as low (less than minus one-half SD of LC signal), medium (more than minus one-half SD to less than one-half SD of LC signal), or high (more than one-half SD of LC signal). InT signals and CR amplitude were unrelated at low LC tone (d = 0.07, p = 0.18) but became more strongly coupled for medium (d = 0.24, p = 8.2e-07) and high tone (d = 0.29, p = 1.2e-05). Combined with our previous finding that CS-evoked LC signals become larger with eyeblink conditioning (J. Lee, H.-J. Boele, and S.S.-H. Wang 2021 Soc. Neurosci. Abstract P510.06), we posit that LC activity regulates InT-driven learned responses on a trial-by-trial basis.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.14

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5R01MH115604-06

Title: Norepinephrine-induced modulation of SK channels in control of synaptic gain of cerebellar Purkinje cells

Authors: *L. SPAETH, H. SNELL, J. TINDI, K. KHODAKHAH;
Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Bronx, NY

Abstract: The cerebellum is essential for accurate motor control and learning. Patients suffering from cerebellar diseases develop mild to severe motor symptoms generally described as ataxia. These symptoms are often transiently aggravated when patients undergo stress or anxiety. Recent evidence from our laboratory shows that stress-induced attacks in Episodic Ataxia 2 are a consequence of a noradrenergic-dependent modulation of SK2 channels through activation of Alpha-1 adrenoceptors on Purkinje cells. In healthy conditions however, noradrenergic modulation of the cerebellar cortex has been shown to improve cerebellar-dependent learning of motor tasks. This project therefore aims to understand the role of norepinephrine in the control of synaptic gain in cerebellar Purkinje cells. Preliminary data obtained in acute cerebellar slices suggests that norepinephrine bi-directionally modulates the gain of granule-cell-to-Purkinje-cell synapses. We hypothesize that alpha and beta-adrenoceptors act in synergy to shape the input/output gain of Purkinje cells and that unoptimized balance may lead to psychogenic stress-induced tremors. Using electrophysiology, optogenetics and behavioral paradigms we aim to address the contributions of different adrenoceptors in the modulation of Purkinje cells activity and how it may contribute to the onset of psychogenic tremors.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

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Program #/Poster #: 639.15

Topic: E.02. Cerebellum

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McKnight Foundation

Title: An activity map of the cerebellum underlying motor planning

Authors: *J. ZHU[1], H. HASANBEGOVIC[2], Z. GAO[2], N. LI[1];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Neurosci., Erasmus MC, Rotterdam, Netherlands

Abstract: The frontal cortex and cerebellum are thought to interact during motor and nonmotor functions (Schmahmann 2019; Ito 2008). The two brain regions are anatomically linked through the pons and thalamus to form cortico-cerebellar loops (Strick 2009). However, it remains

unknown how regions of the two structures form functional cortico-cerebellar loops to mediate specific behaviors. Preparatory activity supporting motor planning is postulated to emerge from distributed processes that involve cortico-cerebellar loops. A localized frontal cortical region in the mouse, anterior lateral motor cortex (ALM), is critical for planning and initiation of directional licking. ALM preparatory activity is dependent on the cerebellum (Gao et al., Nature 2018; Chabrol et al, Neuron 2019). We mapped activity supporting motor planning across the cerebellar cortex and related the activity to mesoscale cortico-cerebellar connectome. Here, we used trans-neuronal anterograde tracing to map ALM inputs to the cerebellar cortex via the mossy fibers. We used monosynaptic rabies tracing to label the Purkinje cells targeting neurons in the fastigial nucleus that projected to the thalamus, which provided the output back to ALM. We surveyed activity across the cerebellar cortex using silicon probe recordings during a delayed response task in which mice used short-term memory to plan directional licking. The anatomy and electrophysiology data were aligned into the Allen Mouse Common Coordinate Framework, facilitating comparisons of the connectivity and activity maps. Contrary to the notion of segregated parallel cortico-cerebellar loops, we found highly divergent and convergent connectivity between ALM and cerebellum. Despite divergent and convergent connectivity, preparatory activity was enriched only in cerebellar regions with conjunction of input-output connectivity to ALM, including parts of crus 1/2, simplex, and vermal lobule 7. Optogenetic perturbation of Purkinje cells in the conjunction regions biased future lick direction. Purkinje cells with only input or output connectivity to ALM did not exhibit preparatory activity and minimally affected the motor planning behavior. These results suggest that Purkinje cells in the conjunction regions selectively amplify inputs from task-relevant neocortical regions and link them with appropriate cerebellar nuclei to form functional cortico-cerebellar loops, and reciprocal communications between the linked regions of neocortex and cerebellum mediate motor planning.

Disclosures: **J. Zhu:** None. **H. Hasanbegovic:** None. **Z. Gao:** None. **N. Li:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.16

Topic: E.02. Cerebellum

Title: Widespread functional convergence from cerebellar cortex to cerebellar nuclei

Authors: *H. HASANBEGOVIC[1], J. ZHU[2], N. LI[2], Z. GAO[1];
[1]Erasmus MC, Rotterdam, Netherlands; [2]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: The cerebellum powerfully influences numerous downstream regions involved in regulation of motor control, reward, and internal brain activity. The output of the cerebellum is funneled through a collection of cerebellar nuclei (CN), namely fastigial, interposed, and dentate nucleus. Purkinje cells in the cerebellar cortex are thought to be organized into parasagittal

modules (medial, intermediate, and lateral) which topographically innervate specific cerebellar nuclei. Here we mapped the cerebellar cortico-nuclear connectivity using photoactivation of ChR2 expressed in Purkinje cells and silicon probe recordings in cerebellar nuclei. Remarkably, photoactivation of a small cluster of Purkinje cells in the dorsal cerebellar cortex was sufficient to silence individual CN. Interestingly, photoactivation mapping revealed only weak topography in the cerebellar cortex to nucleus connectivity. Activation of Purkinje cell clusters anywhere within a 3 mm medial-to-lateral cortical territory significantly inhibited individual nuclei. Similarly, inhibition of Purkinje cell clusters within a 3 mm lateral cortical territory caused significant facilitation in DCN cells. Nevertheless, the strongest Purkinje cell influence to the fastigial nucleus was observed near the vermal regions, and the strongest influence to the dentate nucleus was observed near the lateral regions. Furthermore, pharmacological inhibition of pontine nuclei during Purkinje cell cluster photoactivation diminished the overall suppressing effect on DCN cells. Also, retrograde tracing from individual CN labeled Purkinje cells across a large swatch of the cerebellar cortex, similar to the spatial maps obtained from photoactivation mapping. Our results suggest that individual CN integrate spatially broad input from all three longitudinal compartments, the vermis, the paravermis and the hemisphere. Given diffused mossy fiber input to the cerebellar cortex through pontine nucleus, the broad cerebellar output to the nucleus could allow the cerebellum to learn associations of widespread inputs related to sensory, motor and internal signals with specific teaching signals to form internal models.

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Poster

639. Cerebellum: Interactions With Other Brain Areas

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Topic: E.02. Cerebellum

Support: NINDS: UH3NS100543

Title: A computational model of the dentatothalamocortical circuit to optimize deep brain stimulation for stroke rehabilitation

Authors: *E. BENCE[1], D. ESCOBAR SANABRIA[2], B. CAMPBELL[1], A. G. MACHADO[3], K. B. BAKER[1];
[1]Neurosciences, [2]Biomed. Engin. (Lerner Res. Institute), [3]Ctr. Neurolog. Restoration, Cleveland Clin., Cleveland, OH

Abstract: Preclinical studies with rodents have shown that low-frequency (30 Hz) deep brain stimulation (DBS) of the dentate nucleus improves functional motor recovery after stroke (Cooperrider et al 2020, Baker et al 2010) and informed an ongoing phase 1 clinical trial on DBS for chronic, post-stroke rehabilitation. Optimizing DBS parameters (frequency, pulse-width, amplitude), stimulation patterns (bursts vs. continuous), and strategies (open vs. closed-loop) at a

patient-specific level may be critical to optimizing therapeutic benefit across patients. To better understand how to optimize DBS for stroke rehabilitation, we developed a computational platform that simulates the neuronal dynamics and plasticity of the dentatothalamocortical (DTC) circuit. We created this platform in Simulink (MathWorks, Inc) to leverage existing tools for advanced optimization, signal processing, and feedback control commonly used in automotive and aerospace industries to accelerate the development and validation of similar algorithms. Our computer simulations of the DTC reproduce patterns of neuronal firing recorded from non-human primates and implemented in previously developed computer models (Zhang 2019, Steuber et al 2011). The computer platform presented here will enable us to generate hypotheses on how electrical stimulation reshapes the dynamics of the DTC circuit and how to maximize cortical excitability with DBS for stroke rehabilitation. This platform will also allow us to develop and test the robustness of signal processing, optimization, and closed-loop DBS algorithms aimed to alter neuronal dynamics and plasticity in the dentatothalamocortical circuit before in-vivo testing.

Disclosures: E. Bence: None. **D. Escobar Sanabria:** None. **B. Campbell:** None. **A.G. Machado:** None. **K.B. Baker:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.18

Topic: E.02. Cerebellum

Support: NIH BRAIN Initiative Award 5UH3-NS-10054305

Title: Vigilance modulated cortical evoked potentials generated through stimulation of the dentate nucleus of the cerebellum in post-stroke patients.

Authors: B. A. CAMPBELL[1,2], L. FAVI BOCCA[3], M. SCHROEDEL[3], A. G. MACHADO[3], K. B. BAKER[2];
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Abstract: The ascending cerebellothalamocortical (CTC) pathways are increasingly being explored as potential neuromodulatory targets for the treatment of neurologic and psychiatric disorders; including our recent phase-I trial investigating the effects of dentate nucleus (DN) deep brain stimulation (DBS) on chronic, post-stroke motor impairment. In that trial, DBS was delivered in a continuous, open-loop fashion irrespective of patient state or engagement in rehabilitative efforts. To facilitate the design of future paradigms that maximize engagement of the CTC pathway, we characterized the effect of patient arousal level on the modulatory effect of DN DBS using scalp electroencephalography (EEG). EEG data were recorded from six trial participants who underwent DBS lead implantation in the DN contralateral to the stroke-affected

hemisphere. Cortical evoked potentials (CEP) were generated through time-locked averaging of scalp EEG to low-frequency (<6Hz) stimulation of the DN intra-operatively, under general anesthesia, and during subsequent sessions in the outpatient laboratory under separate eyes-open (EO) and eyes-closed (EC) conditions. The CEP response was quantified through changes in peak to peak components, wavelets, and power spectral density and vigilance in the outpatient setting was classified using Vigall[1] . The CEP was found to vary significantly as a function of patient vigilance. During EO/EC, response characteristics were attenuated as vigilance levels decreased with an almost complete absence under general anesthesia. These results suggest that patient vigilance levels may be an important factor in modulating the dentatothalamocortical circuit and corresponding perilesional cortex. Future motor rehabilitation efforts utilizing DBS of this pathway may benefit from closed-loop approaches incorporating information regarding patient state.

[1] C. Sander, T. Hensch, D. A. Wittekind, D. Böttger, and U. Hegerl, "Assessment of Wakefulness and Brain Arousal Regulation in Psychiatric Research," *Neuropsychobiology*, vol. 72, no. 3-4, pp. 195-205, 2015, doi: 10.1159/000439384.

Disclosures: B.A. Campbell: None. **L. Favi Bocca:** None. **M. Schroedel:** None. **A.G. Machado:** Other; Intellectual property licensed to Enspire DBS. **K.B. Baker:** Other; Intellectual property licensed to Enspire DBS.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.19

Topic: E.02. Cerebellum

Support: R56MH125995
R01MH111868

Title: Cerebellar TMS dosing by cerebellar-cortical and cerebellar-basal ganglia connectivity

Authors: *M. HALKO[1], R. O. BRADY, Jr.[2], L. M. HOLSEN[5], J. XIE[3], W. CHEONG[3], I. LEE[3], U. NAWAZ[3], A. BEERMAN[3], M. NYE[6], S. LAGANIERE[4];
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Abstract: Cerebellar stimulation has a renewed interest for the treatment of psychiatric and neurological conditions. Recent discoveries of motor function within the cerebellum have made understanding the mechanics of cerebellar stimulation imperative. Critically, we have previously demonstrated that cerebellar stimulation impacts network connectivity at rest in acute studies (Halko et al 2014) and across multiple stimulation sessions (Brady et al 2019). Here, we examined if dosage of TMS can be titrated by network connectivity. In a repeated measures

study, 26 healthy received 3 different intensities of intermittent theta-burst simulation determined by active motor threshold (100% aMT, 87.5% aMT, 75% aMT). Stimulation was targeted at the cerebellar vermis representation of the dorsal attention network, with a standard figure of 8 coil, handle facing upward. Functional connectivity at rest and during the gradCPT was collected before and immediately after stimulation. Increasing cerebellar connectivity between default network regions of the cerebellum and default network regions of cortex following stimulation at 100% AMT regions of cortex was observed, but did not reach significance, when compared to 87% and 75% conditions (p=.21). However, cortical network connectivity change between default network and dorsal attention network was strongly linked to attentional performance improvement (r=-0.225, p=0.002). Preliminary analysis suggests that cerebellar-basal ganglia connectivity may be intensity dependent (r=0.19, p=0.07). Overall, our study indicates that titrating the dose of TMS based on motor threshold is an ineffective strategy for cerebellar modulation. We also demonstrate that connectivity change is a required component of behavioral change, suggesting that parameters that can deterministically impact connectivity at the targeted site of intervention may be more relevant considerations for determining effective TMS intensity dosages.

Disclosures: **M. Halko:** None. **R.O. Brady:** None. **L.M. Holsen:** None. **J. Xie:** None. **W. Cheong:** None. **I. Lee:** None. **U. Nawaz:** None. **A. Beerman:** None. **M. Nye:** None. **S. Laganiere:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.20

Topic: E.02. Cerebellum

Support: NIH grant UH3NS100543

Title: Acute dentate nucleus deep brain stimulation modulates corticomotor excitability in chronic stroke

Authors: ***X. LI**[1], K. O'LAUGHLIN[1], Y.-L. LIN[3], K. POTTER-BAKER[4], K. BAKER[2], A. MACHADO[5], E. PLOW[1];
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Abstract: Majority of stroke survivors experience persistent upper limb deficits despite rehabilitation. Brain stimulation has received widespread attention due to its potential to augment effects of rehabilitation. The dentate nucleus (DN) of the cerebellum is considered to be a suitable candidate target to promote functional reorganization of damaged motor networks due to

its excitatory di-synaptic (dentato-thalamo-cortical or DTC) connections to widespread motor cortical regions. However, effects of targeting DN in stroke remain undetermined. We are conducting the first-in-human clinical trial of deep brain stimulation (DBS) targeting DN in persons with chronic stroke (NCT02835443). Here, we report the short-term effects of delivering DN DBS on corticomotor neurophysiology measured using transcranial magnetic stimulation (TMS) to establish proof-of-concept of physiologic changes in DTC. In a double-blinded, cross-over experiment, multiple DBS settings were delivered to 12 chronic stroke survivors with moderate-to-severe upper limb motor impairment. Each DBS setting was delivered at >=1 day interval to ensure washout. TMS was delivered before and after each DBS setting to assess change in corticomotor excitability (active motor threshold, AMT) and output (motor evoked potentials, MEPs at 120% AMT) to paretic finger, wrist, and forearm muscles. Changes in AMT and MEP amplitude were determined for the therapeutic DBS setting selected for the trial vs. sham DBS. Our findings reveal that the therapeutic DBS setting led to a greater reduction in AMT (mean reduction 5.2% MSO, 95% CI [1.2, 9.2]) compared to sham (-0.4% MSO, 95% CI [-1.3, 0.5], $p = 0.013$). MEP amplitude in paretic muscles also increased with therapeutic DBS versus sham ($p = 0.045$). These acute effects of therapeutic DBS were associated with chronic gains in upper limb motor dexterity seen with >=4 months of DN DBS combined with rehabilitation. Participants who showed a larger increase in upper limb motor function tested using the Arm Motor Ability Test also had a larger reduction in AMT with the single session of therapeutic DBS ($r = 0.62$, $p = 0.044$). Overall, DN DBS produces immediate potentiation of corticospinal excitability reflected by a drop in AMT, which is predictive of the degree of motor gains made with long-term DN DBS. The 5.2% MSO reduction in AMT exceeds smallest detectable change value of 1.8 (95% CI [1.0, 2.3]), indicating that these acute effects on corticomotor physiology are beyond what is considered measurement error. TMS can serve as a successful assay to parametrize DBS before chronic long-term treatments, a finding that holds transformative potential for stroke.

Disclosures: **X. Li:** None. **K. O'Laughlin:** None. **Y. Lin:** None. **K. Potter-Baker:** None. **K. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy, Inc.. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy, Inc. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy, Inc., US patent 7,640,063. Methods of treating medical conditions by neuromodulation of the cerebellar pathways. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy, Inc.. **E. Plow:** None.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.21

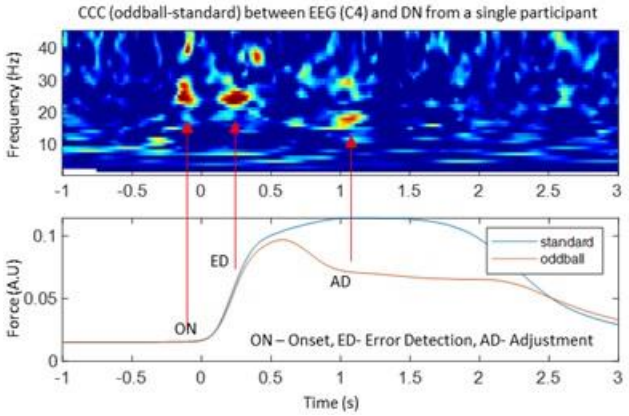
Topic: E.02. Cerebellum

Support: NINDS UH3-NS100543

Title: Cortico-cerebellar interactions during motor error processing in chronic stroke patients

Authors: *R. GOPALAKRISHNAN[1], D. A. CUNNINGHAM[3], A. SRIVASTAVA[2], M. SCHROEDEL[2], K. B. BAKER[2], A. G. MACHADO[1];
[1]Ctr. for Neurolog. Restoration, [2]Neurosciences, Cleveland Clin., Cleveland, OH; [3]Physical Med. and Rehabil., Case Western Reserve Univ., Cleveland, OH

Abstract: The cerebellum is a key structure involved in motor control and is hypothesized to implement a forward internal model that 1) predicts the consequences of motor commands, 2) compares predictions to sensory feedback to generate errors and 3) updates the forward model to improve prediction. Our understanding of the cerebellar role in error processing comes mostly from human metabolic studies and pre-clinical models. As a part of the first in-human phase I trial investigating the effects of deep brain stimulation (DBS) of the cerebellar dentate nucleus (DN) on chronic post-stroke motor rehabilitation, we collected local field potentials from DN and scalp EEG in subjects with middle cerebral artery stroke during a visuomotor oddball task. Six participants performed a response time grip task where a visual Go cue required them to squeeze a dynamometer to move a 2-D ball on a computer display to a target that required either 20% or 40% MVC. On average, 178 randomized trials were performed with 70% of trials at 40% MVC (standard) and 30% of trials at 20% MVC (oddball). Participants were instructed to reach the fixed target, where visual feedback did not indicate if the target was at 20% or 40% of their MVC. As a result, during the oddball trials, participants overshot their target (error) and made adjustment to reach the target. Electrophysiology data from DN and EEG was time-locked to movement onset and processed before computing cortico-cerebellar coherence (CCC) that compared standard vs. oddball trials. Oddball trials exhibited increased CCC in the beta frequency band at pre-movement/onset, error detection and adjustment stages, compared to standard trials. Granger causality showed evidence of bidirectional interaction between ipsilesional cortex and DN. The results show first ever in-human electrophysiological evidence of communication between the DN and ipsilesional cortex during motor error processing. We anticipate that better outcomes can be achieved if we exploit the cerebellar DN role in error prediction toward a closed loop paradigm of DBS that can be used along with motor training.



Disclosures: R. Gopalakrishnan: None. **D.A. Cunningham:** None. **A. Srivastava:** None. **M. Schroedel:** None. **K.B. Baker:** Other; consultants and have intellectual property licensed to

Enspire DBS. Enspire DBS funded part of the clinical trial from which these data are derived but did not fund this research work. **A.G. Machado:** Other; consultants and have intellectual property licensed to Enspire DBS. Enspire DBS funded part of the clinical trial from which these data are derived but did not fund this research work. The authors dec.

Poster

639. Cerebellum: Interactions With Other Brain Areas

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 639.22

Topic: E.02. Cerebellum

Support: NIH (Brain Initiative, UH3NS100543)

Title: Resting state directed cortical and corticocerebellar networks during eyes open and closed conditions in stroke patients

Authors: *B. KRISHNA[1,2], J. BORE-NORTON[1], J. ALMEIDA[3,4], L. FAVI-BOCCA[3,4], K. BAKER[1], A. MACHADO[1,3,4], K. BAKER[1,3];
[1]Neurosciences, Lerner Res. Inst., Cleveland, OH; [2]Biol., Case Western Reserve Univ., Cleveland, OH; [3]Ctr. for Neurolog. Restoration, [4]Neurosurg., Cleveland Clin. Neurolog. Inst., Cleveland, OH

Abstract: Stroke is a disease that leads to altered brain connections and impaired motor function. Our group is investigating whether neuromodulation of the cerebellocortical pathways can enhance chronic motor rehabilitation in stroke recovery. As part of that work, we are characterizing the effect of patient state on connectivity between the two brain regions. *Methods:* We collected electroencephalogram (EEG) and local field potential (LFP) data from patients with eight-channel deep brain stimulation (DBS) leads implanted in the region of the dentate nucleus (DN) during multiple prolonged eyes open (EO) and eyes closed (EC) resting states as an initial proxy for vigilance. The Directed Transfer Function metric was used to characterize network differences in specific frequency bands during the EO and EC states. The power spectral density (PSD) and current source density (CSD) were used to analyze the strength and variability of brain activity, as well as source localization. *Results:* There were significant differences in brain connectivity between the EO and EC states. PSD analysis revealed significant peaks in the EC alpha band, which is consistent with previous studies. Similarly, eyes opening led to a decrease in the source intensities localized in the frontal brain areas as revealed by the cortical source localization performed. The number of nodes and connections were also seen to decrease (particularly in the alpha-band) during EO compared to the EC state. The DN-scalp analysis revealed a decrease in the alpha-band connection strength during the eyes open state, specifically in the frontal regions. We also calculated the differences between the graph network properties in the eyes open and eyes closed states. Specifically, by reconfiguring the DN-scalp network from the EC to EO, there was a decrease in the mean clustering coefficients, the mean shortest path lengths, as well as the local efficiencies in the alpha band implying that a combination of the

DTF and graph theory approaches may be a useful tool for discriminating between the EC and EO conditions and in overall elucidating the changes in DN-scalp functional connectivity which may reflect changes of information processing in the brain. *Conclusion:* We show that there are significant differences in overall connectivity and power of connections with the vigilance state. Differences in the strength of the DN-cerebral cortex connections supports the need for an adaptive, patient-specific approach to neuromodulation of the cerebellocortical pathway that accounts for changes in the strength of functional connectivity in relation to patient state.

Disclosures: B. Krishna: None. **J. Bore-Norton:** None. **J. Almeida:** None. **L. Favi-Bocca:** None. **K. Baker:** None. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy, Inc.. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy, Inc. **K. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy, Inc.. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy, Inc..

Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.01

Topic: E.04. Voluntary Movements

Support: NINDS K12 NRCDG K12NS080223

Title: Differences in movement-related spiking activity in subthalamic nucleus and ventral intermediate nucleus of the thalamus as revealed by an intraoperative reaching task in movement disorder patients

Authors: *R. N. TIEN[1], J. P. PLATT[2], M. MENDLEN[3], D. S. KERN[4], S. G. OJEMANN[1], J. A. THOMPSON[1], D. R. KRAMER[1];
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Abstract: Deep brain stimulation (DBS) is an effective treatment for movement disorders such as Parkinson's disease (PD) and essential tremor (ET). PD, characterized by rigidity, bradykinesia and a resting tremor, is commonly treated by stimulating the subthalamic nucleus (STN), while ET, characterized by an action or postural tremor, is treated by stimulating the ventral intermediate nucleus of the thalamus (VIM). Despite the success of DBS, little is known about the functional properties of individual neurons in these targeted brain areas, partly owing to challenges in recording kinematics in the operating room. To elucidate how the activity of neurons in STN and VIM relates to naturalistic arm movements, we examined single- and multi-unit firing rates obtained while movement disorder patients performed an unconstrained reaching

task during awake DBS surgery. Subjects pointed at a central target and eight radial targets in a center-out-and-back fashion. Movements were recorded with video cameras, and kinematics were extracted offline using DeepLabCut, a deep learning computer vision algorithm. Movement periods were identified using fingertip kinematics. Spiking activity was recorded using microelectrodes, as per standard clinical DBS target mapping procedure. STN activity was obtained from PD patients, while VIM activity was obtained from ET patients. STN units and VIM units displayed different movement-related activity patterns. Across all reaches, significant firing rate modulation was restricted to the time around movement onset in STN. VIM firing rates were also modulated around movement onset but displayed additional modulation during deceleration and stabilization at the target. Regressing firing rates against kinematic parameters revealed that VIM units encoded concurrent movement features more strongly than STN units, and were most closely related to position terms. These results represent a significant step forward in the understanding of STN and VIM in the context of movement disorder pathophysiology. The neural activity patterns identified in this study correspond with the symptomology of PD and ET. Modulation of STN units at movement onset may relate to the resting tremor and difficulty in initiating movements exhibited by PD patients. Modulation of VIM units at the ends of reaches and position encoding may relate to the postural tremor observed in ET. The differential timing of activity in STN and VIM suggests that these areas play distinct and complementary roles in motor control. A deeper understanding of the functional properties of neurons in STN and VIM can guide the development of better treatments for these diseases.

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Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.02

Topic: E.04. Voluntary Movements

Support: CIHR PJT165987

Title: Ventrolateral Prefrontal Cortex Contributes to Human Motor Learning

Authors: N. KUMAR[1,2], A. SIDARTA[1,3], C. SMITH[1], *D. J. OSTRY[1];
[1]McGill Univ., Montreal, QC, Canada; [2]Indian Inst. of Technol., Hyderabad, India; [3]Rehabil. Res. Inst. of Singapore, Nanyang Technological Univ., Singapore, Singapore

Abstract: This study assesses the involvement in human motor learning of the ventrolateral prefrontal cortex (area 9/46v), a somatic region in the middle frontal gyrus. The potential involvement of this cortical area in motor learning is suggested by studies in non-human primates which have found anatomical connections between this area and sensorimotor regions in frontal and parietal cortex and also with basal ganglia output zones. It is likewise suggested by

electrophysiological studies which have shown that activity in this region is implicated in somatic sensory memory and is also influenced by reward. We directly tested the hypothesis that the ventrolateral prefrontal cortex is involved in reinforcement-based motor learning in humans. Participants performed reaching movements to a hidden target and received positive feedback when successful. Prior to the learning task, we applied continuous theta burst stimulation (cTBS) to disrupt activity in 9/46v in the left or right hemisphere. A control group received sham cTBS. The data showed that cTBS to left ventrolateral prefrontal cortex almost entirely eliminated motor learning, whereas learning was not different than after sham stimulation when cTBS was applied to the same zone in the right hemisphere. Additional analyses showed that the basic reward-history-dependent pattern of movements was preserved but more variable following left hemisphere stimulation, which suggests an overall deficit in somatic memory for target location or target directed movement rather than reward processing per se. The results indicate that ventrolateral prefrontal cortex is part of the human motor learning circuit.

Disclosures: **N. Kumar:** None. **A. Sidarta:** None. **C. Smith:** None. **D.J. Ostry:** None.

Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.03

Topic: E.04. Voluntary Movements

Support: NRB118

Title: Pairing Transcranial Magnetic Stimulation and Loud Sounds Produces Plastic Changes in Motor Output

Authors: ***M. GERMANN**[1], N. J. MAFFITT[2], A. POLL[2], M. RADITYA[2], S. J. TING[2], S. N. BAKER[2];
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Abstract: Most current methods for neuromodulation target the cortex. Approaches for inducing plasticity in sub-cortical motor pathways such as the reticulospinal tract could help to boost recovery after damage. In this study, we paired loud acoustic stimulation (LAS) with transcranial magnetic stimulation (TMS) over the motor cortex in healthy humans. LAS activates the reticular formation; TMS activates descending systems, including corticoreticular fibers. Two hundred paired stimuli were used, with 50 ms interstimulus interval at which LAS suppresses TMS responses. Before and after stimulus pairing, responses in the contralateral biceps muscle to TMS alone were measured. Ten, 20 and 30 minutes after stimulus pairing ended, TMS responses were enhanced, indicating the induction of long-term potentiation. No long-term changes were seen in control experiments which used 200 unpaired TMS or LAS, indicating the importance of associative stimulation. Following paired stimulation, no changes were seen in responses to

direct corticospinal stimulation at the level of the medulla, or in the extent of reaction time shortening by a loud sound (StartReact effect), suggesting that plasticity did not occur in corticospinal or reticulospinal synapses. Direct measurements in monkeys undergoing a similar paired protocol revealed no enhancement of corticospinal volleys after the paired stimulation, suggesting no changes occurred in intracortical connections. The most likely substrate for the plastic changes, consistent with all of our measurements, is an increase in the efficacy of corticoreticular connections. This new protocol may find utility, as it seems to target different motor circuits compared to other available paradigms.

Disclosures: M. Germann: None. **N.J. Maffitt:** None. **A. Poll:** None. **M. Raditya:** None. **S.J. Ting:** None. **S.N. Baker:** None.

Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.04

Topic: E.04. Voluntary Movements

Title: Cerebellar Transcranial Evoked Potentials Can Be Reliably Recorded with TMS-EEG

Authors: *D. SPAMPINATO[1], P.-Y. FONG[2], L. ROCCHI[3], J. C. ROTHWELL[4];
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Abstract: Dual site transcranial magnetic stimulation (TMS) allows one to effectively study cerebellar-cortical pathways by pairing magnetic pulses to the cerebellum and motor cortex. While the studies utilizing this strategy have helped to understand some of the neurophysiological processes of cerebellar-M1 connectivity in the context of motor control and cerebellar-dependent motor learning, this technique relies on examining motor evoked potentials that suffer from large trial-by-trial variability. This is likely due to MEPs containing a mixture of both cortical and spinal components. Thus, developing alternative methods to index cerebellar-cortical connectivity, not dependent on the excitability of spinal circuits, may provide more detailed physiological insights into these pathways. The present study aimed to investigate the cerebellar output projection to the neocortex by using TMS in combination with electroencephalography (EEG). First, we tried to identify a reliable cortical response produced by single-pulse cerebellar TMS in a resting state. We arranged auditory control and somatosensory control experiments to investigate how these sensory inputs affect the brain when giving cerebellar TMS. After the successful removal of components related to auditory input produced by the large double-cone TMS coil, we found that the cerebellar TMS-evoked potential (TEP) was different from the activity evoked by somatosensory input. Moreover, we also found the cerebellar TEP was quite consistent and mirrored scalp distribution when separately stimulating the two cerebellar hemispheres. In a second experiment, we investigated whether the

amplitude of the cerebellar TEP would be sensitive to state-dependent changes in cerebellar activation that occur during arm-reaching visuomotor adaptation task. Our cerebellar TEP demonstrated the reproducible results of a frontal-positive peak at 80ms post-TMS (P80) increased after learning (e.g. comparing post-baseline vs post-adaptation TMS-EEG responses). The change in P80 following complete adaptation might be related to the increased activity in the state of the dentatothalamocortical track and the cerebellar-prefrontal networks that play a key role in learning new sensorimotor transformations. Overall, we demonstrate that cerebellar TEPs can be isolated artifacts and are reproducible across different groups of subjects when an adequate coil, stimulation intensity, and careful artifact processing are achieved. The results of the second experiment provide evidence that TMS-EEG can be used to measure the state of the cerebellar-prefrontal connectivity in the context of motor learning.

Disclosures: D. Spampinato: None. **P. Fong:** None. **L. Rocchi:** None. **J.C. Rothwell:** None.

Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.05

Topic: E.04. Voluntary Movements

Support: ANPCyT, FONCyT: PICT-2019-02156
ANPCyT, FONCyT: PICT-2018-01150

Title: Immediate post learning sleep improves motor memory retention and promotes the fast spindle-SO coupling over the contralateral motor network

Authors: *A. SOLANO[1], L. A. RIQUELME[1], D. PEREZ-CHADA[2], V. DELLA-MAGGIORE[1];
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Abstract: The last decade has seen remarkable progress in the identification of neural markers of sleep-dependent memory consolidation. Yet, most advances have emerged from the field of declarative memory. Recently, however, we have shown that the level of coupling between fast spindles and slow oscillations (SO), a mechanism involved in the consolidation of declarative memories (Muehlroth et al., 2019), is modulated by visuomotor adaptation (VMA), a type of procedural learning. Specifically, VMA increases the spindle-SO coupling, over the contralateral hemisphere to the trained hand, and this increment predicts overnight memory retention (Solano et al., 2021, 2022). Here, we deepen on the mechanisms involved in sleep-dependent consolidation of motor memories by manipulating the time interval elapsed between training and sleep, a procedure that has been effective in unveiling the benefit of sleep in declarative tasks (Backhaus et al., 2008; Talamini et al., 2008; Payne et al., 2012; Sawangjit et al., 2018). Two

groups of subjects were trained ~14 hours (T-14h; n=23) or ~10 minutes before sleep (T-10min; n=23), and EEG was recorded overnight. Memory retention was assessed 24 h and 2 weeks after learning. We predicted that if sleep modulates the consolidation of VMA memories, sleep immediately after training should improve overnight memory retention and increase spindle-SO coupling locally. We found that the temporal proximity between training and sleep enhanced memory retention by 30% (main effect of group, p=0.049), a phenomenon that persisted for at least two weeks. In line with the observed gains in performance, the density of fast spindles increased during NREM sleep over the hemisphere contralateral to the trained hand (p=0.002). Critically, the coupling between fast spindles and SO significantly increased in the T-10min group compared to the T-14h group (p=0.019). These results are in line with the Systems Consolidation Hypothesis. The proximity between learning and sleep did not influence the delta band activity early during NREM sleep, suggesting that the Synaptic Homeostasis Hypothesis (SHY) may not explain behavioral gains induced by this manipulation. Our results provide evidence in favor of a common mechanism in the stabilization of declarative and motor memories.
Refs: Muehlroth et al.(2019) Sci Rep. 9(1):1940; Solano et al., (2021) Cer.Cortex; Solano et al., (2022) Front.Neurosci. vol. 16; Backhaus et al. (2008) Neurobiol. Learn. Mem. 89(1); Talamini et al.(2008). Learn. and Mem., 15(4); Payne et al. (2012)PLoS ONE, 7(3); Sawangjit et al. (2018) Nature. 564(7734)

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Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.06

Topic: E.04. Voluntary Movements

Support: CIHR PJT-389243

Title: Motor learning by observing synthetic videos depicting force field learning

Authors: *N. MANGOS[1,2], M. IYAYI[1,2], G. HUANG[1,2], P. L. GRIBBLE[3,2,1];
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Abstract: Motor learning typically involves extensive physical practice; however, like physical practice, watching another person learn a motor skill can also drive an adaptation of observers' force production patterns. Little is known about how visual information about another individual's movement kinematics is transformed into neural representations of dynamics that are then used to alter motor function. Here we asked what properties of the observed stimuli are necessary for this transformation to occur. To determine whether this effect depends on vision of

a real human, we measured participants' force generation patterns before and after watching a video in which an animated, human-like figure recreated the movements of a real human learning to reach in a force field. We found that the animation successfully induced dynamic adaptation in observers. In a second experiment, we tested whether such adaptation could also be induced using a video in which there was no visual representation of a human at all; only point-and-line representations of reach trajectories were shown. We found that observers still adapted to produce forces that were specific to the force field in the video. We demonstrate that neural representations of novel dynamics can be acquired by observing biological motion, even when there is no human depicted. This finding suggests that future studies of observation-related changes in limb control might be able to use entirely synthetic stimuli to induce sensorimotor plasticity and learning in humans. We tested this idea in a third experiment, where we assessed adaptation after observers had watched a video depicting simulated kinematics of a computational model of a human arm learning to reach in a force field. The use of synthetic videos in future research could allow for flexible manipulation of the observed stimuli to test hypotheses about the mechanisms underlying observational motor learning, as well as to develop observation-based tools for neurorehabilitation that can be customized to meet the needs of individual patients and target specific deficits for recovery.

Disclosures: **N. Mangos:** None. **M. Iyayi:** None. **G. Huang:** None. **P.L. Gribble:** None.

Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.07

Topic: E.04. Voluntary Movements

Support: National Science Foundation 1553895

Title: Motion-dependent motor learning based on explicit visual feedback demonstrates less spatial generalization and temporal stability than adaptation to physical perturbations

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Abstract: Adaptation of arm reaching movements to physical perturbations (e.g., a velocity-dependent force-field, vFF) is thought to be a largely implicit learning process. We have recently shown that subjects can also learn the motion state-dependent changes to motor output induced by vFFs based on explicit feedback; subjects are shown visual information on the extent the applied temporal force pattern matches the required velocity-dependent force profile if the vFF perturbation had been applied. Here, we examined the spatiotemporal properties of this learning compared to adaptation to physical vFF perturbations. There were two subject groups and two experimental paradigms. All subjects made 10 cm reaching arm movements between two targets

using a robotic *manipulandum*. One group (n=40) experienced physical vFF perturbations while the second (n=40) was given explicit visual feedback (eVF) of the required force-velocity relationship. In the latter, subjects moved in force channels and we provided visual feedback of the lateral force exerted during the movement, as well as the required force pattern based on the movement velocity. Twenty subjects from each group completed one of two experiments. In the first paradigm (Spatial generalization), following vFF or eVF training, generalization of learning was tested by requiring subjects to make movements to 14 target locations (0^o, $\pm 7.5^o$, $\pm 15^o$, $\pm 30^o$, $\pm 45^o$, $\pm 75^o$, $\pm 135^o$ and 180^o around the trained location). In the second paradigm (Temporal stability), following training we examined the decay of learning over 8 delay periods (0, 3, 6, 10, 20, 30, 60, or 120 seconds). Results showed that learning based on eVF did not generalize to untrained directions while the generalization for the vFF was significant for targets $\leq 45^o$ away from the trained direction. In addition, the decay of learning for the eVF group was significantly faster than the vFF group (a time constant of 2.38 ± 7.98 sec vs 9.87 ± 6.45 sec). Our results suggest that learning based on explicit information demonstrates less spatial generalization and temporal stability than adaptation to physical perturbations. Future studies will combine eVF and vFF to probe learning properties when provided both types of information (i.e., concurrently utilizing both learning mechanisms).

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Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.08

Topic: E.04. Voluntary Movements

Title: The influence of transcranial alternating current stimulation of primary motor cortex on overhand throwing performance

Authors: R. BOSS[1], M. I. PREMYANOV[1], K. J. NOORDA[1], E. WILKINS[1], D. AYNLENDER[1], R. DAVIDSON[2], M. PANTOVIC[1], T. HAGANS[3], Z. A. RILEY[4], *B. J. POSTON[1];
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Abstract: Transcranial alternating current stimulation (tACS) applied to the primary motor cortex (M1) has been shown to improve performance in relatively simple motor tasks. However, no tACS studies have examined complex, multi-joint tasks involving whole-body coordination. The purpose was to determine the influence of tACS on motor skill acquisition and motor learning over multiple days in a complex overhand throwing task. The study utilized a randomized, between-subjects, SHAM-controlled, double-blind experimental design. Sixteen

young adults (12 men, 4 women) were allocated to either a tACS or a SHAM group. Three experimental sessions were completed on consecutive days and each session involved overhand throwing trials to a target in a baseline-test block, 5 practice blocks, and a post-test block (10 trials per block). tACS was applied to M1 during the practice blocks for 20 minutes (current strength: 1 mA, frequency: 70 Hz). SHAM stimulation was applied according to standard SHAM protocols. Motor performance was quantified as the endpoint error, which was the primary dependent variable. Endpoint error was analyzed with a 2 *Group* (tACS, SHAM) x 3 *Day* (1,2,3) between-subjects ANOVA. The *Group* x *Day* interaction and *Group* main effect were both non-significant ($P = 0.939$ and $P = 0.688$, respectively). The main effect for *Day* ($P = 0.635$) was also non-significant despite the 7.1% overall reduction in endpoint error (SHAM = 8.6%; tACS = 5.5%), due to the high inter-individual performance variability. Both groups improved endpoint accuracy with practice, but the improvements did not reach statistical significance. Most importantly, the findings indicate that application of tACS over 3 days of practice does not improve motor learning in an overhand throwing task to a greater extent than practice alone in young adults. Thus, different stimulation parameters, longer-term (weeks/months) stimulation, and large sample sizes may be needed to observe an improvement motor learning in complex motor tasks due to tACS in young adults.

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Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: 640.09

Topic: E.04. Voluntary Movements

Title: When task variability becomes too high: boundaries of structural learning in a visuomotor task

Authors: *F. A. KAGERER[1], A. T. BRUNFELDT[2];
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Abstract: Structural learning is described by faster learning in situations that share characteristics of the previously experienced context, as shown in visuomotor adaptation tasks (Braun et al, 2009). Studies also indicate that it seems to be afforded by explicit learning processes (Bond and Taylor, 2017). In most of these visuomotor paradigms, participants reach sequentially to 8 targets with the same rotation in hand feedback applied to each trial; they are then exposed to a new rotation for the next block of 8 trials, etc. The question thus arises to what extent the explicitness postulated to underlie structural learning is modulated by the number of repetitions of one particular rotation angle. To test this, we had three groups (n= 13, 11, 12) of

right-handed college-aged participants (mean age: 22.3 years, SD: 4.7) learn a visuomotor structure by reaching from a starting position to two targets at 90 or 270 degrees over 240 trials with a variable rotation in hand feedback using the Kinarm endpoint lab. During a training phase, we exposed groups to either two, four, or eight consecutive trials with the same rotation before applying a new, randomly generated rotation. All groups then performed a 16-trial washout period with veridical visual feedback (a control group (n= 10) performed under veridical visual feedback only). This was followed by a test phase, during which the groups, after 16 baseline trials with veridical visual feedback, were exposed to 40 trials of a fixed 60-degree visual feedback perturbation, again followed by 16 trials with veridical feedback. Our preliminary results show that measures like initial directional error (IDE, angular difference between required and actual hand movement direction at peak velocity, reflecting planning) and RMSE (distance between hand trajectory and straight movement vector from peak velocity onward to movement termination, reflecting feedback processes) showed benefits only for the 8-repetition group who had had a significantly faster learning rate over the first 16 trials of the test phase compared to the other groups, as reflected by IDE ($F(2, 43) = 4.70$, $p < 0.01$). This benefit remained for the rest of the adaptation period; aftereffects were not different between groups. These results indicate a lower limit for repetition within the variable perturbation schedule in order for structural learning to be beneficial; this might be mediated by making it more difficult to exploit explicit learning processes as the number of repetitions decreases.

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Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: 640.10

Topic: E.04. Voluntary Movements

Support: Craig H Neilsen Foundation
Shirley Ryan AbilityLab Accelerator Program

Title: Gamified Electromyographic Biofeedback with Wearable Sensors for People with Tetraplegia

Authors: *R. COTTON[1,2];
[1]Shirley Ryan Abilitylab, Chicago, IL; [2]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL

Abstract: People with tetraplegia consistently rank recovery of arm function among their highest priorities to improve independence and quality of life. Intensive, repetitive practice can stimulate motor recovery through activity-dependent plasticity and generally produces a greater benefit with greater dosage, but we lack the tools to help patients perform enough high-quality practice at home. Electromyographic biofeedback has been shown to improve muscle control for subjects

with incomplete spinal cord injury, and even produce functional improvements, but typically specialized lab equipment and personnel. We aim to overcome these limitations by developing an affordable, wearable sensor platform that can be employed by subjects for gamified biofeedback therapy. We developed a wearable, Bluetooth-connected electromyography (EMG) sensor platform with accompanying smartphone software that enable people with SCI to perform EMG biofeedback. We also developed games controlled by muscle activity recorded with these sensors. The games adapt their difficulty to the maximal volitional electromyographic activity detected to encourage participants to increase their muscle activation. We tested the system on participants with tetraplegia for both single sessions and over several sessions. Leveraging the portable nature of the system, these tests were performed in numerous locations including hospital rooms, hallways, and a laboratory. Activity from multiple paretic muscles in the arms including the triceps, wrist extensors and wrist pronators was acquired with the sensors and subjects could control the games, even without detectable movement. For the majority of sessions and muscles, maximal volitional electromyographic activation increased following biofeedback therapy, consistent with previously published results without gamification. Compliance for multiple sessions was high. Participants responded positively to the game experience and reported the therapy felt like a good workout. Gamified electromyographic therapy provided via wearable sensors is feasible for people with tetraplegia, can be conveniently performed anywhere, participants responded to it positively, and the increases in muscle activation seen with laboratory-based approaches were replicated. This may enable people with tetraplegia to independently perform similar therapy at home. Future work will be required to demonstrate if biofeedback therapy results in functional improvements in arm function.

Disclosures: R. Cotton: None.

Poster

640. Motor Learning: Neurophysiology

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.11

Topic: E.04. Voluntary Movements

Title: Steadiness training with concentric or eccentric contractions: effects on ankle movement control and single leg balance

Authors: M. WEINTRAUB, J. HUBBARD, S. DELMAS, J. KIM, Y. CHOI, B. YACOUBI KEYHANI, ***E. CHRISTOU**;
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Abstract: Movements are accomplished through an interaction of muscle shortening (concentric) and lengthening (eccentric) contractions. There is strong evidence that concentric contractions have better movement control than eccentric contractions due to a distinct neural activation of muscle. To date, only one study examined the effect of steadiness training through lifting and lower light loads with the index finger and found improvements in motor control and

manual dexterity. However, the individual effectiveness of steadiness training interventions performed with either concentric or eccentric contractions to improve motor control remain unexplored. The purpose of this study was to compare concentric and eccentric steadiness training performed with the left ankle, on the ability of young adults to control ankle dorsiflexion movements with the right and left ankle and balance on one leg. This abstract is based on preliminary findings from 3 participants. We aim to recruit 30 healthy young adults (18-35 years) that will be randomly assigned into one of three groups (CONTROL, CON, ECC). The CONTROL group did not train, whereas the CON and ECC groups participated in 6 sessions of concentric or eccentric steadiness training with the left ankle. Each participant performed a pre- and post-test 2 weeks apart that included the following tests: 1) tracing a spatiotemporal target (15º ROM in 7.5 s) with the left and right ankle as accurately and as steady as possible by controlling the tibialis anterior muscle with concentric and eccentric contractions; 2) balance on a single leg (left and right) for 30 s. Each training session comprised of 30 tracing trials of a spatiotemporal target (15º ROM in 7.5 s). The CON group traced the target with concentric contractions of the tibialis anterior (TA), whereas the ECC group traced the target with eccentric contractions of the TA. Online visual feedback of the ankle position relative to the spatiotemporal target was given during the training session and pre- and post-tests. Our preliminary findings show that the individual who participated in the CON group exhibited greater improvements in movement control of the trained leg (left) and balance on one leg (left or right) compared with the individual in the ECC or CONTROL groups. The individual who participated in the ECC group exhibited greater improvements in movement control of the untrained leg (right) compared with the individuals in the other two groups. Although premature, these findings suggest that concentric training is best suited for improving balance activities, whereas eccentric training is best suited for transferring to the contralateral limb.

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Poster

640. Motor Learning: Neurophysiology

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Program #/Poster #: 640.12

Topic: E.04. Voluntary Movements

Support: VA ORD Award I01RX002619-01A2

Title: Baseline Corticomotor Excitability Predicts Motor Skill Performance and Neuroplastic Response to Exercise in Aging Adults

Authors: *T. NOVAK[1], J. NOCERA[1,2], K. MCGREGOR[3], L. KRISHNAMURTHY[1,4,2], K. MAMMINO[1], M. BELLO[1], G. CHAMPION[5];
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Abstract: A growing body of literature suggests that 1) exercise may help to mitigate the changes in inhibitory-receptor function due aging, and 2) a relation exists between exercise-induced changes in intracortical inhibition (ICI) and improved motor dexterity outcomes in older adults. However, to date, there is a lack of consensus on the functional significance of these adaptations in the context of adaptive motor skill acquisition. In the present study, 18 healthy older adults (mean=70 ±5 years) were randomly assigned to a 12-week exercise intervention focusing on either strength/balance (n=9) or aerobic (n=9) training. Differential training effects on motor learning and cortical inhibition (ICI) were then assessed using serial reaction-time/accuracy performance and paired-pulse transcranial magnetic stimulation (ppTMS) measures, respectively. There were no significant differences between training groups in terms of ICI and motor performance change after intervention, as both groups showed increased cortical inhibition and improved motor performance outcomes after intervention ($p < .05$). A subsequent analysis of variance was then performed comparing ICI response to exercise according to whether individuals exhibited intracortical inhibition (ICI: n=7) or facilitation (ICF: n=11) during baseline TMS assessments. Significant group x session interactions were observed, as individual's who showed baseline ICF exhibited a significant shift towards ICI, and the ICI exhibited a non-significant shift towards ICF, regardless of their assigned exercise condition (all $p < .05$). Moreover, regression analysis showed a significant inverse relation between baseline ICI/ICF measures and reaction time performance during the initial motor assessment session (all $R^2 >= .63$). Individuals who showed greater baseline intracortical facilitation demonstrated better initial motor skill performance. While preliminary, these results suggest that aging-related reductions in ICI may reflect an adaptive process that can may account for individual response outcomes to behavioral interventions. This work may inform research in risk stratification and non-invasive brain stimulation (NIBS) to promote precision medicine.

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Poster

640. Motor Learning: Neurophysiology

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Topic: E.04. Voluntary Movements

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Title: Reconfiguration of human cortico-subcortical manifold structure during motor reinforcement learning

Authors: *Q. NIK, D. GALE, C. ARESHENKOFF, A. DE BROUWER, J. NASHED, J. WAMMES, J. SMALLWOOD, J. GALLIVAN;
Queen's Univ., Kingston, ON, Canada

Abstract: Successful reinforcement learning depends on the coordinated activity of multiple neural systems distributed across cortex and subcortex. Although we generally understand the role of the striatum and medial prefrontal cortex in reward- and value-related processes, we currently lack a more comprehensive picture of how whole-brain, cortico-subcortical systems function in an integrated manner when learning new mappings between motor commands and reward feedback. To address this gap, we studied human brain activity using functional MRI (fMRI) during a motor reinforcement learning task, wherein subjects learned to shape their reach trajectories purely through reward-based and not error-based directional feedback. By projecting patterns of cortical and subcortical functional connectivity onto compact low-dimensional manifold spaces, we estimated the relative positions of brain regions along this cortico-subcortical space and how they traverse as a function of learning. Relative to baseline trials, during learning we find that areas in ventromedial prefrontal cortex exhibit significant contraction along the cortico-subcortical manifold, which is associated with their increased covariance with areas in sensorimotor cortex and anterior medial temporal cortex. At the same time, we also find that areas in orbitofrontal cortex exhibit significant expansion along the manifold, which is associated with their increased covariance with areas in ventral striatum and posterior cingulate cortex. We further relate aspects of these learning-dependent patterns of manifold contraction versus expansion to differences in subject-level performance in the task. Together, our findings provide a unique characterization of the landscape of functional cortico-subcortical changes associated with motor reinforcement learning and suggest that such learning involves the integration of sensorimotor brain regions with higher-order brain areas in transmodal cortex.

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Poster

640. Motor Learning: Neurophysiology

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NSERC RGPIN-2019-04440, D.S.M.

Title: The effects of sleep deprivation on motor learning in young adults

Authors: D. GILL[1], A. ROENNINGEN[1], J. MUGLISTON[1], J. WANG[4], A. LANG-HODGE[2], D. F. COOKE[2], A. R. MCINTOSH[2], D. S. MARIGOLD[2], ***B. KENT**[3];
[1]Psychology, [2]Biomed. Physiol. and Kinesiology, [3]Simon Fraser Univ., Burnaby, BC, Canada; [4]Rotman Res. Inst., Univ. of Toronto, Toronto, ON, Canada

Abstract: Sleep deprivation can have detrimental effects on learning and memory. The present study aims to examine whether sleep following learning affects next-day performance on two novel motor learning tasks in healthy young adults. The first is a digitized mirror tracing task that asks participants to view a shape on a laptop and then trace the shape with a digital marker on a graphic drawing tablet. The second is a stepping task that asks participants to predict where a moving target will stop and step to the center of the predicted location from a standing position. The target is projected on the ground and moved in specific patterns before stopping. Within each testing session, each movement pattern is repeated (interleaved, pseudorandom order), allowing participants to learn the trajectories. Position sensors are placed on the participant's shoe to determine foot placement and stepping accuracy. We are recruiting participants between 18 - 40 years old, who are in good general health, with no known visual or sleep disorders. Both the sleep deprived group (recruitment target n=16; females = 8) and the rested control group (recruitment target n=16; females = 8) wear actigraphy watches for the duration of the study and complete sleep diaries each day. The sleep deprived group is observed overnight in the laboratory to ensure a full night of sleep deprivation, while wearing UVEX S1933X blue-wavelength blocking glasses, to minimize phase-shifting effects of nighttime light exposure. The rested control group sleeps in their own home before returning to the laboratory for an assessment of their performance the next day. It is hypothesized that those in the rested group will demonstrate superior performance on the motor tasks compared to the sleep deprived participants. Preliminary results show that the participants who are sleep deprived (n=7) show improved performance on the motor learning tasks, suggesting that sleep is not necessary for learning these motor learning tasks. Data collection and analysis is ongoing. The present study will serve as a pilot to inform future research on the role of sleep in motor learning.

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Poster

640. Motor Learning: Neurophysiology

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Program #/Poster #: 640.15

Topic: E.04. Voluntary Movements

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NSF Graduate Research Fellowship

Title: Simplified internal models in human control of complex objects

Authors: *S. BAZZI[1], S. STANSFIELD[2], N. HOGAN[2], D. STERNAD[1];
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Abstract: Numerous studies in motor neuroscience provided evidence that humans have internal models of their body to predict and compensate for the forces imposed by the environment. Going beyond the typical reaching paradigm, a few studies that examined manipulation of non-rigid objects also showed that humans relied on an accurate internal model of the object. However, these studies only considered linear mass-spring systems with relatively simple dynamics. Can humans acquire models of more complex objects with nonlinear and potentially chaotic dynamics? What level of detail do they represent? To answer these questions, participants physically interacted with a nonlinear underactuated system mimicking a cup of sloshing coffee: a cup with a ball rolling inside. The cup and ball were simulated in a virtual environment and subjects interacted with the dynamical system via a haptic robotic interface. Participants were instructed to move the system and arrive at a target region with both cup and ball at rest, 'zeroing out' residual oscillations of the ball. To create interesting dynamics, a metronome paced the movements to be faster than the ball's period of oscillation. 11 participants completed 4 blocks, 50 trials in each block. As the task was challenging, subjects first practiced the required pace in block 1; in block 2 they focused on how to 'zero out' terminal ball oscillations; in blocks 3 and 4, subjects aimed to both minimize ball oscillations and move at the required pace. The data displayed two robust features: 1) cup velocities exhibited two unequal peaks, 2) the velocity minimum between the two peaks increased for faster movements. To probe human control, we conducted simulations with 'input shaping', a control strategy that applies a series of timed pulses to move a dynamic object from point to point with no residual oscillations. Since the timings and amplitudes of these pulses depend on the controller's model of the object, input shaping served as a tool to identify the human's internal representation of the cup-and-ball. Five control models with varying internal representations were compared against the human data. The models ranged from a detailed representation of the coupled hand and nonlinear cup-and-ball system to a rigid-body model without oscillatory dynamics of the ball. Results showed that the features in the data were correctly predicted by a simple internal model that represented the cup-and-ball as a single rigid mass coupled to the hand impedance. These findings provide evidence that humans use simplified internal models to control and manipulate complex objects.

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Poster

640. Motor Learning: Neurophysiology

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Title: WITHDRAWN

Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.17

Topic: E.04. Voluntary Movements

Support: 418589

Title: The effect of accumulated learning history in a strictly controlled implicit visuomotor task

Authors: *E. DE LA FONTAINE[1], L. ARSENAULT-LÉVESQUE[1], J.-F. LEPAGE[2], P.-M. BERNIER[1];
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Abstract: The sensorimotor system shows an impressive ability to update motor commands following changes in sensory contexts. This process is known as implicit adaptation (Krakauer et al., 2019) and is driven by sensory prediction errors. Recent evidence suggests that adaptation to a particular perturbation is attenuated if it is preceded by prior exposure to that same perturbation in a close temporal frame, a form of anterograde interference (AI; Avraham et al., 2021; Hamel et al., 2021). It has been suggested that this may be due to either saturation of recently overactivated synapses (Keck et al., 2017) or the presence of a washout period between learning sessions (Kitago et al., 2013). None of the previous studies fully isolated the effect of accumulated adaptation without a washout period in which participants knowingly restore their initial motor behaviors. The aim of this study was to test whether an accumulated learning history attenuates subsequent implicit adaptation, as measured with a post-rotation bias (PRB) protocol. In a counterbalanced and within-subjects design, two conditions (Control and Adaptation) were conducted over the course of two visits in which participants (n = 10; preliminary results; targeted sample n = 30) performed reaching movements toward one of three targets during a First Session (180 trials). In the Adaptation condition, participants unknowingly adapted to gradual visuomotor rotations that continuously changed between ± 4°. In the Control condition, no rotation was introduced. Hand direction at peak velocity (HDPV) was used as the main dependent variable. After a 2-min break, participants again performed reaching movements (180 trials) with a visuomotor rotation on ± 30° on 33 % of the trials. PRB of the HDPV was measured on the subsequent unperturbed trial, in which participants were instructed to ignore the rotation. Analysis of the longitudinal data across the six phases confirmed that participants adapted to the gradual rotations in the Adaptation condition as attested by significant differences in HDPV over the 6 phases, whereas HDPV did not change during the Control condition (p = 0.001, dz = 0.670, and p = 0.230, dz = 0.180, respectively). Analysis of the Second Session revealed significant PRBs in directions opposite to the induced rotations. However, the

magnitude of the mean difference (MD) in PRB between conditions was not significantly different (MD = 0.237°, $p = 0.262$, dz = 0.379). Similarly, no difference was observed in the kinematic data (RT and MT; MD < 5.619, $p > 0.09$, dz < 0.685). Overall, these results suggest that implicit adaptation as measured by the PRB method is not constrained by an accumulated learning history.

Disclosures: E. De La Fontaine: None. **L. Arsenault-Lévesque:** None. **J. Lepage:** None. **P. Bernier:** None.

Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 640.18

Topic: E.04. Voluntary Movements

Support: Penn State Dorothy Foehr Huck and J. Lloyd Huck Endowment for Kinesiology and Neurology

Title: Lateralization of learning and interlimb-transfer of direction but not distance learning in 2D shuffleboard skilled tasks.

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Abstract: Learning a new motor task with one arm can transfer to improve performance and learning with the other arm. However, transfer of task performance is most often incomplete, and the pattern of transfer is influenced by motor lateralization. Many previous studies have addressed transfer in adaptation tasks, such as visuomotor rotation and curl field learning. We now investigate whether lateralized control mechanism influences retention and interlimb transfer in a motor skill task. We designed a virtual 2d shuffleboard task in which the participant uses a cursor (as a paddle) to hit a puck toward a target. The direction the puck is determined by the direction of the cursor velocity vector component that passes through the center of the puck at impact. The distance of the puck is determined by the amplitude of this vector component together with a virtual friction coefficient. We modified this task to two subtasks: a direction task and an amplitude task. In the direction task, participants were required to control cursor direction while control of impact amplitude was not constrained. In the amplitude task, participants were required to control impact amplitude but not direction. In each task, participants were randomly assigned to two groups that has different arm orders, and they practiced the task for two consecutive days. They switched the arm in the last session of day 2. Based on the dynamic dominance hypothesis, we predicted that direction control should be influenced by motor lateralization, while distance control should not. Our data in the distance task showed symmetry in the extent and time course of initial learning, as well as retention. Most significantly, our

findings revealed symmetrical transfer between the arms. In contrast, the direction task data showed asymmetry in learning and transfer. Initial learning was faster in the dominant arm. In this task, we found complete transfer from the non-dominant arm to the dominant arm, but incomplete transfer in the opposite direction. We conclude that the pattern of interlimb transfer of skill learning depends on whether the task recruits lateralized control mechanisms. Similar to previous findings in visuomotor rotation transfer, trajectory direction control transfers asymmetrically with greater transfer to the dominant arm. In contrast, distance or amplitude control is not lateralized and shows symmetrical transfer. Most significantly, both of our tasks showed similar time courses of learning, suggesting that they reflected similar levels of task difficulty.

Disclosures: J. Yuk: None. **J.B. Dingwell:** None. **R.L. Sainburg:** None.

Poster

640. Motor Learning: Neurophysiology

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Program #/Poster #: 640.19

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Dorothy Foehr Huck and J. Lloyd Huck Distinguished Chair Endowment Fund

Title: Anodal (excitatory) HD-tDCS demonstrates a contralateral role of the left posterior parietal cortex in interlimb transfer of learning

Authors: *B. DEXHEIMER[1], C. SANDBERG[2], K. SELBY[1], S. A. JAYASINGHE[3], S. S. KANTAK[4], R. L. SAINBURG[1,5];
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Abstract: Motor learning is critical for recovery of function after stroke. However, the functional neuroanatomy underlying motor learning remains poorly understood. We have previously provided support for a lateralized role of the left posterior parietal cortex (PPC) in motor learning, revealing visuomotor adaptation deficits for *both* arms following left, but not right, PPC lesions. Visuomotor adaption disruptions have also been demonstrated in a neurologically intact population using inhibitory high-definition transcranial direct current stimulation (HD-tDCS) to the left PPC. In the present study, we predicted that excitatory HD-tDCS to left PPC of healthy, neurologically intact participants would facilitate visuomotor

adaptation and interlimb transfer of learning. Sixty neurologically intact participants completed a visuomotor rotation task in a 2D virtual environment. Participants were randomized to receive either sham stimulation (n = 20), left PPC stimulation (n = 20), or right PPC stimulation (n = 20). Following a baseline period, participants adapted to a 30-degree visuomotor rotation (adaptation phase) with their dominant arm, after which the reaching arm was switched to assess interlimb transfer of learning to the non-dominant arm. Performance was quantified using initial direction error, reflecting feedforward mechanisms of movement planning, and final position error, reflecting a final steady-state position. We observed no effect of stimulation on learning, as represented by no difference in all performance metrics between the groups during the adaptation phase. Comparing groups for interlimb transfer of learning, we observed positive effects of left PPC stimulation for initial direction error (feedforward mechanisms), while also demonstrating negative effects of left PPC stimulation for final position error (error correction mechanisms). No difference in each performance metric was observed between the right PPC and sham stimulation groups. This pattern of findings is reflective of previous research in our laboratory showing that left and right PPC lesions have differential effects on initial direction and final position errors. When the rotation was removed, the left PPC group demonstrated significantly larger aftereffects when compared to the right stim and sham groups. These findings suggest a lateralized role of the left PPC for interlimb transfer from the dominant to non-dominant arm.

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Poster

640. Motor Learning: Neurophysiology

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Title: The ipsilateral role of left posterior parietal cortex in interlimb transfer of visuomotor learning: An anodal HD-tDCS study

Authors: *N. M. KITCHEN[1,2], B. DEXHEIMER[2], C. W. SANDBERG[3], K. SELBY[2], R. L. SAINBURG[2,1];
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Abstract: The ability to learn and adapt movements is an important feature of motor control, providing us with the ability to compensate for changes in our environment and physiology, yet

the central neuroanatomy underlying motor learning is still not well understood. Previously, we have shown that learning to adapt reach movements to altered visual feedback is disrupted in *both* arms for individuals with left, but not right, posterior parietal cortex (PPC) lesions. Likewise, impairments in visuomotor adaptation, and the transfer of learning between limbs, has also been reported for young, neurotypical adults receiving inhibitory (cathodal) high-definition transcranial direct current stimulation (HD-tDCS) to the left-PPC. Yet the potentially beneficial effects of anodal (excitatory) left-PPC stimulation on visuomotor learning are still unclear. We recently examined this with young, right-handed, neurotypical adults who received 20min of stimulation while making targeted reaches with their dominant hand, first under veridical (baseline) and then 30deg rotated (adaptation) visual feedback conditions. This was followed immediately by adaptation with the non-dominant hand to assess interlimb transfer of learning. Although there were no performance differences between groups (left-PPC, right-PPC or sham) during the initial adaptation phase, left-PPC stimulation resulted in greater transfer effects to the untrained, non-dominant arm of (1) lower initial direction errors (reflecting feedforward mechanisms) but with (2) larger final position errors (reflecting feedback correction mechanisms) than other groups. These findings are consistent with the reciprocal increase of initial direction errors and decrease of final position errors seen in *both* hands with lesions to left-PPC, yet it remains unclear whether excitatory left-PPC stimulation has a bilateral effect too. In other words, is the *ipsilateral* role (train non-dominant left hand, transfer to dominant right) for anodal left-PPC HD-tDCS similar or different to the *contralateral* role (train dominant right hand, transfer to non-dominant left) we found previously. We investigate this line of work and discuss these findings in the context of neural lateralization of motor function and potential application of anodal left-PPC HD-tDCS for supplementing post-stroke functional recovery.

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Poster

640. Motor Learning: Neurophysiology

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Program #/Poster #: 640.21

Topic: E.04. Voluntary Movements

Support: NIH T32 HD007414 to AJB
NIH R35 NS122266 to AJB

Title: Motor learning by reinforcement matures throughout childhood

Authors: *N. M. HILL[1,2], L. M. MALONE[1,2], H. M. TRIPP[1,2], D. M. WOLPERT[3], A. J. BASTIAN[1,2];
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Abstract: Learning by reinforcement is characterized by a willingness to explore the workspace and incorporate success and failure feedback in order to select the subsequent actions that maximize reward. While age related differences in reinforcement learning have been shown in the cognitive domain, less is known about the developmental progression in the motor domain. We designed a novel, home computer-based "Penguin Game" where participants used a mouse, trackpad, or touchscreen to move a cartoon penguin with the goal of getting across the ice without slipping. Continuous movement data were collected at 10-70 Hz remotely on a web-based interface. Learning was driven by a binary reward signal: if movement was successful, a short cartoon video played and if unsuccessful, a sad face appeared and the video did not play. This reinforcement-based task addresses two questions: 1) can children explore the workspace to find the location that maximizes reward when given binary feedback, and 2) how do children respond to success and failure on a trial by trial basis? The game included different task blocks were the reward for movement of the penguin was determined based on: 1) whether the penguin hit a discrete target at varied locations, 2) an unseen position-based probability gradient within a wide target; the gradient landscape contained a 100% reward zone (width matching discrete targets) flanked by decreasing reward probabilities, or 3) clamped feedback of all success or all failure independent of movement location. A cohort of 40 children, ages three to 17 years, and 40 young adults, ages 18 to 35 years, completed the task. All participants, regardless of age, achieved high success rates in the blocks with discrete targets indicating appropriate motor control to accurately hit a target. In the gradient block, reward rates ranged from 60 to 98% for the majority of adults and children over nine years old, while rates for younger children dropped as low as 31%. These results demonstrate a developmental component to incorporating feedback that is distinct from the motor control required to move the game piece. In the failure clamped block, as expected, most participants in both groups increased their trial-to-trial variability indicating exploration in response to failure. For younger children who were less responsive to the reward gradient, the increase in exploration in response to repeated failure may indicate that a higher threshold of failure is required to change behavior. Overall, this demonstrates that even the youngest children are able to incorporate success and failure feedback to drive a change in movement.

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Poster

640. Motor Learning: Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: 640.22

Topic: E.04. Voluntary Movements

Support: NIH R35 NS122266

Title: Reinforcement-based learning of a walking task with multiple solutions.

Authors: A. BAKKUM[1,2], J. STENUM[1,2], R. T. ROEMMICH[1,2], A. J. BASTIAN[1,2];
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Abstract: Walking on a new terrain, such as an icy sidewalk, often requires exploration to learn an appropriate stepping pattern. During learning, many patterns may be explored to find one that best accounts for constraints (e.g., slipperiness, your footwear, or if you are in a hurry). In this example, reinforcement signals from slips versus stable steps may drive exploration and learning of a successful pattern. Here, we tested this in the laboratory by asking whether people could explore and learn new walking patterns in response to binary visual feedback. Healthy adult participants walked on a tied-belt treadmill and were given a reward signal (exploding fireworks) on a large screen if their stride length (i.e., right + left step length) fell within an unseen reward window. Because a stride is the sum of 2 consecutive steps, many patterns of right + left step lengths could result in a reward. In baseline, the unseen reward window was set to their preferred stride and subjects tended to take equal right and left steps with high reward rates. During learning, we shifted the unseen reward zone to determine if a reinforcement signal could shape participants behavior to both longer (20% increased) and shorter (20% decreased) stride lengths (counterbalanced design). Here again, many solutions could lead to reward. Longer strides could be achieved through different patterns of lengthening either one or both steps, and vice versa for shorter strides. We found that our participants learned ~70% of the desired long shift and retained ~55% during a retention block. In contrast, they learned ~45% of the desired short shift and retained ~38%. We suspect that subjects learned the long shift better because they had increased time to plan the longer versus shorter strides (~1.36s vs. ~1s). They also used an asymmetric stepping strategy for the long shift by increasing their second (left) step more than their first (right) step. The short shift was achieved by maintaining right and left step length symmetry. Taken together, our findings demonstrate that people can shape their walking patterns in response to binary visual feedback over short periods of time (within 100 strides). Importantly, the strategy used when exploring different step-length combinations depends on the constraints of the task.

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Poster

640. Motor Learning: Neurophysiology

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Topic: H.08. Learning and Memory

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Title: Learning-related contraction of grey matter in rodent sensorimotor cortex is associated with adaptive myelination

Authors: *T. MEDIAVILLA[1], Ö. ÖZALAY[1], H. M. ESTÉVEZ-SILVA[1], B. FRIAS[2], G. ORÄDD[1], F. R. SULTAN[1], C. BROZZOLI[3], B. GARZÓN[3,4], M. LÖVDÉN[3,4], D. J. MARCELLINO[1];
[1]Umeå Univ., Umeå, Sweden; [2]Univ. Col. London, London, United Kingdom; [3]Karolinska Inst., Stockholm, Sweden; [4]Univ. of Gothenburg, Gothenburg, Sweden

Abstract: From observations in rodents, it has been suggested that the cellular basis of learning-dependent changes, detected using structural magnetic resonance imaging (MRI), may be increased dendritic spine density, alterations in astrocyte volume, and adaptations within intracortical myelin. Myelin plasticity is crucial for neurological function and active myelination is required for learning and memory. However, the dynamics of myelin plasticity and how it relates to morphometric-based measurements of structural plasticity remains unknown. We used a motor skill learning paradigm to evaluate experience-dependent brain plasticity by voxel-based morphometry (VBM) in longitudinal MRI, combined with a cross-sectional immunohistochemical investigation. Whole brain VBM revealed non-linear decreases in grey matter (GM) juxtaposed to non-linear increases in white matter (WM) that were best modelled by an asymptotic time course. Using an atlas-based cortical mask, we found non-linear changes with learning in primary and secondary motor areas and in somatosensory cortex. Analysis of cross-sectional myelin immunoreactivity in forelimb somatosensory cortex confirmed an increase in myelin immunoreactivity followed by a return towards baseline levels. The absence of significant histological changes in cortical thickness further suggests that non-linear morphometric changes are likely due to changes in intracortical myelin for which morphometric WM volume (WMV) data significantly correlated with myelin immunoreactivity. Together, these observations indicate a non-linear increase of intracortical myelin during learning and support the hypothesis that myelin is a component of structural changes observed by VBM during learning.

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Poster

640. Motor Learning: Neurophysiology

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Program #/Poster #: 640.24

Topic: B.09. Glial Mechanisms

Support: NIH F32NS110481
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Title: Assessing the computational power of astrocyte Ca2+ transients in the motor cortex during learning

Authors: *J. SHIH[1], G. T. DRUMMOND[2], C. DELEPINE[1], N. M. LE[2], Y.-N. LEOW[2], M. SUR[3];
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[3]Dept. of Brain and Cognitive Sci., MIT Grad. Brain and Cognitive Sci., Cambridge, MA

Abstract: Astrocytes are the most abundant glial cell type in the brain. Increasing evidence shows that they respond to and influence neuronal function at the synaptic, cellular, and network levels, and that this relationship may be reflected in transient astrocytic calcium (Ca2+) activity. These Ca2+ transients are spatiotemporally diverse, which may indicate complex bidirectional astrocyte-neuron interactions that drive learning and behavior. We have previously shown that correlated neuronal activity in the motor cortex is associated with learning and hypothesize that astrocyte Ca2+ signaling is critical for the temporal specificity of neuronal activity during behavioral epochs. To test this, we measured astrocytic Ca2+ during motor skill learning and reinforcement learning paradigms, and manipulated Ca2+ activity using DREADDs. Our preliminary data show that astrocyte Ca2+ signals are increased during motor execution and that the slower temporal scale of astrocyte activity relative to neuronal activity allows for task optimization. Disruption of astrocyte Ca2+ signals alters neuronal ensemble formation and learning. Using support vector classifiers and generalized linear models, we show that astrocyte Ca2+ signals can be used to decode behavioral outcomes and encode task parameters, suggesting that astrocyte Ca2+ is a critical part of cortical information processing during learned behaviors.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 641.01

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 NS053603
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Title: Preserved neural covariance patterns across multiple unconstrained behaviors

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Abstract: Our brain needs to coordinate many components when we make even simple movements in a complex environment. Here we ask whether population activity in primary motor cortex (M1) encodes only the action, or its context as well. We previously showed that covariance patterns among neurons in the hand area of primate M1 are well preserved across a range of trained hand and wrist movements when the animal is confined to a primate chair, suggesting that the neural representations of these movements are task-invariant (Gallego et al., 2018). In contrast, a separate study observed dramatically different M1 covariance patterns in mouse forelimb motor cortex for a single-limb precision pulling task and treadmill walking (Miri et al., 2017). We wondered whether these different observations were due to the species difference, or whether the constraint of a primate chair restricted the range of covariance patterns observable in M1. Considering that treadmill walking was naturalistic, while precision pulling needed extensive training, it is also possible that the similarities in M1 covariance patterns for the monkeys occurred because they were both well trained behaviors. To approach these questions, we set up a large telemetry cage which allowed monkeys to move freely. We wirelessly recorded both M1 spiking activity and electromyogram signals from muscles in the monkey's arm and hand, and classified monkeys' behaviors from synchronized videos. These behaviors included interacting with an instrumented power grasp device, picking treats with a precision grasp, and crawling over perch bars spanning the width of the cage mounted 10 cm above the floor. We then quantitatively evaluated the similarity between the neural covariance patterns across different behaviors. We found a large fraction of neural covariance was preserved when the monkey's hand was used to grasp perch bars while crawling and when taking treats or grasping the instrumented device, even though muscle activation patterns and body postures changed considerably between these conditions. This finding indicates that, in contrast to rodents, M1 neurons in primates seem to exhibit a relatively fixed covariance pattern even across movements made in quite different behavioral context. This study extends our previous findings from constrained, in-lab tasks (Gallego et al., 2018) to unconstrained conditions where the monkey needs to coordinate multiple extremities and body postures. As the power grasp task required significant training, while treat retrieval and crawling over the perch bars did not, these findings also suggest that both trained and naturalistic behaviors share similar neural covariance patterns.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 641.02

Topic: E.04. Voluntary Movements

Support: MWK Lower Saxony ZN3422 “DeMoDiag”
DFG CRC-1528 “Cognition of Interaction”

Title: Spatio-temporal patterns of Macaques’ foraging trajectories in an experimentally controlled naturalistic terrain

Authors: N. SHAHIDI, Z. AHMED, I. LACAL, A. M. GAIL;
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Abstract: Foraging animals are adapted to sparse and uncertain natural environments. While sparsity drives the animals to optimize energy consumption, uncertainty drives them to balance food foraging with information-seeking. The foraging strategy of macaque monkeys has been widely studies when making repeated binary decisions between concurrently presented options. Although this paradigm is effective in revealing decision policies in restrained setups, it is highly abstracted from natural foraging in which food is distributed in space and decisions are based on the spatio-temporal structure of the environment. Here we ask if balancing food and information seeking with energy consumption explains the navigation trajectory of a foraging monkey in a naturalistic terrain. In our new Exploration room Platform, we developed a controlled environment in which monkeys freely forage hidden food within a matrix of spatially distributed piles across the terrain. We recorded their behavior using 6-8 cameras, allowing precise 3-dimensional reconstruction of their posture from which we identified their actions. We observed that monkeys travel longer distances before searching the next pile, when they do not find food in the current pile. In contrast, finding food is tallied by short steps on a convoluted path. Moreover, monkeys’ success rate was higher when searching a recently encountered map of food, compared to when they were first exposed to the same map. In a modelling approach, we were able to produce equivalent behaviors using canonical agents that maximized their food and information gain while minimized their locomotion. These agents represented the environment using spatio-temporal kernels, as they navigated through a sequence of piles. Using only three types of spatial kernels, food availability, information availability, and proximity, the locomotion trajectory of a simulated agent resembled the trajectory of a monkey in that the traveled distances were shorter after finding food. We clustered the searched piles based on whether the monkey ‘stayed’ nearby after searching the pile or ‘left’ the area to search a pile further away. For monkeys as well as the simulated agent, the size of a cluster was positively correlated with the total amount of food in that cluster. For the simulated agent, the correlation diminished, if the information availability kernels were weighed substantially higher/lower than the food availability kernels. Taken together, our results suggest that a canonical model with a low number of spatial kernels can explain core statistical properties of monkeys’ foraging trajectories in an environment with spatially structured food abundance maps.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 641.03

Topic: E.04. Voluntary Movements

Title: Associations between movement sequence memory and spatial ability in actor-combatants

Authors: *B. BROCKSHUS, E. L. STEGEMOLLER;
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Abstract: Current models of human memory focus on auditory and visual information with less regard for kinesthetic information. A sample of 24 actor-combatants, practitioners of stage combat, was recruited. Movement-related memory was assessed with forward and reverse span tasks using large body movements. Language-related memory was assessed using forward and reverse digit span tasks. Visuospatial ability was assessed using a two-dimensional shape rotation task and a paper folding task. Associations were found between movement short-term memory and mental rotation (Spearman rho=0.41, p=0.044), movement working memory and mental rotation (Spearman rho=0.46, p=0.024), and movement working memory and spatial visualization (Spearman rho=0.48, p=0.017). Planned human movement may be supported by visuospatial processes. Thus, arts-related movement experiences may promote spatial ability and vice versa. This is particularly relevant considering the importance of spatial ability for success in science, technology, engineering, and mathematics (STEM) education and careers.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

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Program #/Poster #: 641.04

Topic: E.04. Voluntary Movements

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Title: Enhanced interpersonal coordination in the military - Evidence from synchronized motor performance and hyperscanning EEG

Authors: *N. BOURGUIGNON[1], N. COUCKE[2], E. CASPAR[3], S. LO BUE[1];
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Abstract: Many military activities require precise interpersonal coordination to ensure their safety and efficiency. Simultaneous shooting of a given target, or so-called ‘Vic’ formation flights, illustrate the requirement for skillful motor synchronization in military operations. A relevant question therefore is whether military selection and training enhances cooperation skills in service soldiers compared to civilians and what the neurocognitive mechanisms are that underlie variations in interpersonal cooperation? The present study addressed these issues by

comparing the performance of 40 cadets from the Belgian Royal Military Academy and 48 age-matched civilian controls in a computer-based cooperation game while their EEG was simultaneously recorded through hyperscanning. The paradigm involved pairs of subjects engaging in patrolling scenarios that required reaching checkpoints simultaneously or in sequence depending on the checkpoints' color. Performance was assessed in two conditions: (1) a SOLO condition where subjects patrolled on their own, and (2) SYNC[HRONIZED] condition where subjects had to coordinate their actions to reach the same checkpoint together or one after the other. Scenarios where subjects failed to synchronize their actions in the SYNC condition were aborted and had to be started over. Success rate was measured by the average number of times a scenario had to be replayed and the average time spent on a given scenario before completion. Results reveal that military subjects were significantly more successful and faster at completing the trials than civilian subjects in the SYNC condition, confirming a significant impact of military training and selection on their motor synchronization skills. To elucidate what neurocognitive mechanisms drive this effect, average inter-subject coherence in the five major frequency bands (alpha, beta, theta, gamma and delta) was calculated from the subjects' EEG and compared between groups. Enhanced cooperation in military subjects was associated with significant inter-subject coherence in the gamma band, while civilian subjects' EEG featured greater inter-subject coherence in the beta band. These differences are interpreted in terms of distinct attentional strategies: While civilian subjects might cooperate through top-down attentional control over their motor output, making them slower and more error-prone, military subjects might predominantly rely on bottom-up, input-driven attention and shared situational awareness. These findings have important implications for research aimed at explaining the neurocognitive underpinnings of interpersonal cooperation.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 641.05

Topic: E.04. Voluntary Movements

Support: NINDS Grant R01-NS-104898

Title: Two dimensional spatial structure in correlations across sensorimotor cortex of freely behaving marmosets

Authors: *P. L. APARICIO[1], J. WALKER[2], A. F. TORTOLANI[1], N. G. HATSOPOULOS[1]; [2]Dept. of Organismal Biol. and Anat., [1]Univ. of Chicago, Chicago, IL

Abstract: The correlation of neural signals across brain areas has been used to identify functional networks or large-scale brain circuits whose coordinated activity is believed to be critical in understanding how the brain processes information. Here we report the identification

of 2D spatial structure in the pairwise correlations of local field potentials (LFP) recorded from a 4x4mm area in the sensorimotor cortex of the unrestrained and freely behaving marmoset. We recorded wirelessly from a 96 channel Blackrock Utah array during the day while the animal engaged in unconstrained spontaneous behavior, goal-directed foraging, and prey capture, and during nighttime sleep. LFP signals were low pass filtered (at 250Hz) and downsampled at 1KHz from each electrode. We quantified the spatial structure by fitting a two parameter (decay rate and baseline) exponential function to the average pairwise correlation values from discrete distances across the spatial extent of the array. As expected, correlations in the LFP signal fell off with distance from the reference electrode, reaching a baseline value ~1.2mm on average. The correlation values were not static and fluctuated dynamically showing significant changes in the baseline of the exponential fit across time in both the awake and sleep recordings. Additionally, the decay rate across time was faster in the awake recordings, suggesting differences in the dynamic coordination of neural activity between the two states. Finally, we observed reliable spatial clusters, or 2D spatial patterns, in the pairwise correlations across the array. These clusters could be identified independently both in sleep recordings and during awake behavior. During daytime activity, increases in the spatial correlations coincided with voluntary foraging epochs of behavior.

Disclosures: P.L. Aparicio: None. **J. Walker:** None. **A.F. Tortolani:** None. **N.G. Hatsopoulos:** F. Consulting Fees (e.g., advisory boards); BlackRock Microsystems, Inc..

Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

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Program #/Poster #: 641.06

Topic: E.04. Voluntary Movements

Support: R01-NS104898

Title: Understanding sensorimotor control through marmoset prey capture

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Abstract: Adaptive control of movement is fundamental to the success of an organism. Prey capture provides an integrative behavior to study flexible, visually guided sensorimotor control. To build a phenomenological model of common marmoset (Callithrix jacchus) prey capture, we use deep learning based computer vision tools (Mathis et al. 2018) to quantify the kinematics of both marmoset hand and moths. We find that moth evasive behavior may push the limits of the marmoset's pursuit abilities. While distributions of marmoset hand and moth speeds are largely overlapping (median reaching speed 6.15 cm/sec, median prey speed 8.79 cm/sec), the maximum speeds per episode for the moth exceed the maximum speed the marmoset achieves in pursuit

(max reaching speed mean: 65 cm/sec; max prey speed mean 94 cm/sec). We use the geometry of moth trajectories to evaluate the extent to which moth flight can be predicted, finding that the autocorrelation of moth velocities decays to zero in all three spatial dimensions by 135 msec. This epoch encapsulates the bulk of the distribution of best fit sensorimotor delays across the marmoset's visuomotor system which we estimated using a mixed guidance model (Brighton & Taylor 2019) fit to both marmoset hand and moth trajectories. Notably, the mixed guidance model fails to capture instances when the marmoset does not engage in continuous pursuit of the moth but rather brings the hand back to the torso between epochs of pursuit. While the model assumes perfect knowledge of the feedback control variables guiding pursuit, these variables are only available to the marmoset through noisy channels of vision and proprioception where sensorimotor errors likely accumulate with sequential movements. We conclude by presenting a kinematic/psychometric marmoset model as a step toward approximating egocentric gaze informing the computation of feedback control variables guiding marmoset pursuit behavior.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

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Program #/Poster #: 641.07

Topic: E.04. Voluntary Movements

Support: R01 NS113071
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Title: Motor cortical dynamics during vocal production in the Singing Mouse

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Abstract: Vocal interactions, such as during a conversation, require considerable behavioral flexibility. Similarly, in the Alston's singing mouse (S. teguina), vocal partners can take part in fast and flexible counter-singing in which two animals coordinate their ~10 s songs with silent gaps of ~500 ms. Recently, we discovered that a cortical locus in the singing mouse, the orofacial motor cortex (OMC), is crucial for coordinated vocal interactions; inactivation of this region abolished counter-singing behavior (Okobi*, Banerjee*, et al., 2019). However, the circuit dynamics within the OMC and their impact on vocal production remained unclear. To address this issue, we used chronic high-density silicon probe recordings in OMC (n = 396 neurons, 5 animals) of male singing mice. During singing, we found that neural ensembles displayed reliable activity that was distinct from that recorded outside of the context of song.

Further analysis revealed structured spiking activity on two behaviorally relevant timescales. About 35% of recorded neurons have slowly (~ seconds) varying persistent dynamics that - as a population - track the initiation, progression, and termination of songs. Leveraging the large trial-by-trial variability in motor output (songs), we found that this persistent activity in individual neurons “stretches” or “compresses” in accordance with the overall song durations. A second population (~30%) exhibited spiking that was temporally phase-tuned to individual song notes. Further analyses of spike times revealed that this fast-timescale modulation has a small time-lag with respect to song notes, fitting the profile of sensory feedback signals. To understand the impact of motor cortical dynamics on song production, we next constructed two models in which the OMC outputs are either (1) completely determining the structure of the songs, including the articulation of constituent notes or (2) working hierarchically with a downstream pattern-generator capable of shaping note structure. Each model generates behavioral predictions that we compare against both normal songs as well as songs after focal cooling of OMC. We find support for the second model, in which the duration of vocal bouts is controlled by OMC using a temporal scaling mechanism, while the structure of individual notes is determined downstream. Taken together, we describe a strategy by which motor cortical activity, acting via downstream pattern-generator circuits, generates behavioral (vocal) flexibility. We provide a systems-level framework to study hierarchical motor control, a challenge faced by natural and artificial agents moving through the world.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

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Program #/Poster #: 641.08

Topic: E.04. Voluntary Movements

Support: BRAINS R01-MH12275101
R21-MH127501
McDonnell Center for Systems Neuroscience

Title: High-density diffuse optical tomography (HD-DOT) for measuring neural responses to gross motor imitation

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[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Kennedy Krieger Inst., Baltimore, MD; [3]Johns Hopkins Univ., Baltimore, MD; [4]Univ. of Nottingham, Nottingham, United Kingdom

Abstract: High-density diffuse optical tomography (HD-DOT) for measuring neural responses to gross motor imitation

Current evidence suggests that children with autism spectrum disorder (ASD)—a neurodevelopmental disorder characterized by social-communication deficits and restricted/repetitive behaviors—have impaired gross motor imitation abilities. However, determining neural signatures of gross motor imitation has been challenging due to limitations in current imaging methods. Functional magnetic resonance imaging (fMRI), considered the gold standard in functional neuroimaging, is extremely sensitive to motion artifacts and requires participants to lay in a supine position incompatible with overt motion. In contrast, high-density diffuse optical tomography (HD-DOT) produces maps of brain function comparable to fMRI in an open scanning environment better-suited for overt, gross motion. Establishing the feasibility of using HD-DOT during motor imitation will allow us to assess neural signatures associated with imitation abilities significant for social development and interactions, including in children with or at risk for ASD. In this study, we collected HD-DOT data from twenty adults aged 21-31 while they performed motor observation and imitation tasks, along with standard functional localizer tasks. Consistent with prior literature, we observed activations in visual and superior temporal areas during motor observation and imitation, with additional activations in the primary and supplementary motor areas during motor imitation. The contrast of imitation>observation revealed stronger responses in the premotor, primary motor, and superior temporal cortical areas, in correspondence with previous research. Given these results, we conclude that HD-DOT is a suitable modality for measuring neural responses to motor observation and imitation in a sample of healthy adults. Future directions include correlating neural responses with imitative abilities and expanding this research into children with and without ASD.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

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Topic: E.04. Voluntary Movements

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McCausland Center for Brain Imaging Seed Grant

Title: Effect of positive social comparative feedback on resting state connectivity of dopaminergic neural pathways: a preliminary investigation

Authors: *A. F. LEWIS, R. BOHNENKAMP, M. MYERS, D. DEN OUDEN, S. FRITZ, J. C. STEWART;
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Abstract: Positive social comparative feedback, or feedback that indicates to the learner that they are performing better than others, is hypothesized by the OPTIMAL theory to trigger a dopaminergic response in the brain that benefits motor learning. Dopamine is a neurotransmitter that signals when a positive outcome or reward might occur from an action, a process critical to learning. Dopamine is synthesized in two midbrain regions, the ventral tegmental area (VTA) and substantia nigra (SN), and operates along several pathways including the mesolimbic, mesocortical, and nigrostriatal. However, no studies have investigated the effect of positive social comparative feedback on dopaminergic pathways in the brain. The purpose of this preliminary study was to determine the effect of positive social comparative feedback on the resting state connectivity of dopamine pathways. Thirty individuals (mean age 25.6 ± 4.3; 21 females) practiced a joystick-based motor sequence task over a single session (28 blocks for a total of 140 sequence repetitions). Participants were divided into two feedback groups: RT ONLY and RT+POS. The RT ONLY group received feedback about their actual response time to complete the sequences, while the RT+POS group received feedback about their actual response time plus an indication that they were faster than others (i.e., positive social comparative feedback). Twelve minutes of resting state functional magnetic imaging were collected before and after motor practice, and seed-based connectivity analyses determined resting state connectivity between brain regions along dopamine pathways (VTA-nucleus accumbens, VTA-orbitofrontal cortex, SN-caudate, SN-putamen). Both groups improved performance over practice (main effect of block on response time, $p < 0.001$). However, the RT+POS group showed faster response times ($p < 0.001$) and higher peak velocities ($p < 0.001$) than the RT ONLY group. Resting state functional connectivity increased between VTA and left nucleus accumbens (mesolimbic pathway) for the RT+POS group ($p = 0.001$; Cohen's $d = 1.03$, large effect) but not for the RT ONLY group ($p = 0.281$; Cohen's $d = 0.29$, small effect). Neither group showed significant changes along the mesolimbic or nigrostriatal pathway. These results support the OPTIMAL theory prediction that positive social comparative feedback may trigger a dopaminergic response in the brain, specifically along the mesolimbic dopamine pathway. Overall, this study is an initial step in investigating how practice conditions can be altered to target dopamine pathways, a pathway that is critical to motivation and learning.

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Poster

641. Sensorimotor Mechanisms in Naturalistic and Social Behavior

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 641.10

Topic: E.04. Voluntary Movements

Title: Action observation combined with proprioceptive stimulation shapes cortical plasticity

Authors: *M. BOVE, A. BISIO, M. BIGGIO, L. AVANZINO, L. BONZANO;
Univ. of Genoa, Univ. of Genoa, Genoa, Italy

Abstract: Physical practice is crucial to evoke cortical plasticity, but motor cognition techniques, such as action observation (AO), have shown their potentiality in promoting it when associated with peripheral afferent inputs, without the need of performing a movement. Here we investigated whether the combination of AO and proprioceptive stimulation, based on muscle tendon vibration at 80Hz, able to evoke a kinaesthetic illusion (KI) of movement, induced plasticity in the primary motor cortex (M1). Further, inter and intra-individual variability are major factors that may weaken the effectiveness of neural plasticity-inducing conditioning protocols. For these reasons, we also investigated a possible predictor of plasticity for the AO-KI protocol. In particular, we evaluated M1 excitability changes during the administration of the AO-KI protocol and we investigated the relationship between M1 excitability changes during the stimulation and the occurrence of neuroplasticity phenomena. Forty five healthy subjects participated to two experimental sessions: 1) Before / After AO-KI (B/A-AO-KI), when AO-KI was administered, and recruitment curves (RC) were measured before, immediately, 30 and 60 minutes after the stimulation to evaluate plastic changes in M1excitability by means of TMS; 2) DURING-AO-KI (D-AO-KI) and DURING KI (D-KI), when M1 excitability was evaluated during the administration of the combined stimulation or during the mere proprioceptive stimulation, respectively; AO-KI consisted of 25 paired stimulations during which subjects observed a thumb-flexion movement and simultaneously received a proprioceptive stimulation able to induce a kinesthetic illusion of a thumb-flexion movement. During KI participants received the same intermittent mechanical vibration protocol as during AO-KI. Motor evoked potential (MEP) amplitude was recorded from abductor pollicis brevis. As a result, M1 excitability significantly increased in D-AO-KI and D-KI, but the augmentation was significantly higher in D-AO-KI with respect to D-KI. Furthermore, the higher the increment in MEP amplitude in D-AO-KI with respect to rest, the greater the increase in M1 excitability in B/A-AO-KI, as measured by changes in the RC slopes in all the post evaluation epochs. These results support the existence of a summation between AO and KI afferent signals visible in the higher increment in M1 excitability during the combined stimulation. Furthermore, the present findings suggested that being highly responsive to the stimulation facilitates the development of motor cortical plasticity changes.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

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Topic: E.05. Brain-Machine Interface

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ERC-2019-PoC BRAINGAIT 875660

Title: Natural control of lower limb movements using a brain spine interface

Authors: *H. LORACH[1,2], A. GALVEZ[1,2], V. SPAGNOLO[1,2], N. INTERING[2,3], F. MARTEL[4], S. A. KOMI[1], R. DEMESMAEKER[1,2], C. HARTE[1,2], S. KARAKAS[4], T. AKSENOVA[4], O. FAIVRE[4], V. AUBOIROUX[4], F. SAUTER-STARACE[4], S. CHABARDÈS[5], G. CHARVET[4], J. BLOCH[1,2,3], G. COURTINE[1,2];
[1]Ctr. for Neuroprosthetics and Brain Mind Institute, Swiss Federal Inst. of Technol. (EPFL), LAUSANNE, Switzerland; [2]Defitech center for interventional neurotherapies (.NeuroRestore), Lausanne, Switzerland; [3]Lausanne Univ. Hosp. (CHUV), Lausanne, Switzerland; [4]Univ. Grenoble Alpes, CEA, LETI, Clinatec, F-38000, GRENOBLE, France; [5]Univ. Grenoble Alpes, Grenoble Univ. Hospital, F-38000, GRENOBLE, France

Abstract: Introduction: Spinal cord injury interrupts the communication between the brain and the spinal cord, having a devastating impact on motor control and sensory function. In most severe cases SCI leads to complete paralysis having a dramatic human, societal and economical cost. A digital bridge to restore communication between the brain and the lumbosacral region of the spinal cord that produces walking can in principle restore walking function and promote neurological recovery in people with chronic paraplegia. We tested this hypothesis by developing a fully-implantable Brain-Spine Interface (BSI) in a chronically paralyzed participant. **Methods:** In the context of the STIMO-BSI study (NCT04632290) we implemented the BSI in an individual with chronic tetraplegia (Incomplete lesion at C5-C6). We implanted (1) a pair of WIMAGINE® devices (64 electrocorticographic electrodes) that enables safe long-term wireless recordings over the sensorimotor cortex, and (2) a stimulation system which translates the decoded intentions from electrocorticographic signals into analog modulations of epidural electrical stimulation targeting the region of the spinal cord involved in walking. **Results:** The brain signals were streamed in real time to a computing unit that uses iterative classification algorithms to generate online predictions of the motor intentions. We were able to classify motor attempts for the different joint movements (hip, knee and ankles bilaterally) with high accuracy. The decoded predictions were converted into electrical stimulation commands delivered by the implantable pulse generator via the epidural lead targeting the different dorsal roots of the leg muscles. The participant used the BSI system over a 40-session rehabilitation period and we evaluated the effect of the BSI system with clinical outcomes which shows functional recovery. **Conclusion:** A BSI system can be used as a neuroprosthesis to restore communication between the brain and the spinal cord below the lesion. This digital bridge that converts thoughts into actions enables an individual with chronic tetraplegia to walk naturally in community settings and can be used as a neurorehabilitation therapy to promote neurological recovery.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

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Swiss National Science Foundation NCCR Robotics
ERC-2019-PoC BRAINGAIT 875660

Title: Outstanding stability of ECoG signals recorded epidurally with the WIMAGINE implants in a context of BCI clinical trials

Authors: *F. SAUTER-STARACE[1], C. LARZABAL[1], S. BONNET[2], L. STRUBER[1], A. GALVEZ[3,4], F. MARTEL[1], T. AKSENOVA[1], O. FAIVRE[1], V. AUBOIROUX[1], H. LORACH[3,4], J. BLOCH[3,4,5], G. COURTINE[3,4], G. CHARVET[1], S. CHABARDÈS[6];
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Abstract: Introduction: In the framework of two clinical trials involving participants with chronic spinal cord injury, we intend to take advantage of the remaining brain motor activity to drive effectors to compensate motor handicap. To this aim, we developed WIMAGINE a fully implantable battery-less device able to record ECoG signals on 64 channels through the duramater.In BCI & tetraplegia clinical trial (NCT02550522), tetraplegic participants are trained to drive an exoskeleton to walk in the lab or to reach and grasp objects from the daily life whereas in STIMO-BSI clinical trial (NCT04632290), a paraplegic participant is also implanted with a spinal neurostimulator to elicit motor activity of their lower limbs. **Results**: When we started the design of the WIMAGINE implant, there was a great challenge on such a Brain

Computer approach based on epidural signals. Indeed, the signals might have been too weak or their quality might have lessened dramatically after several months due to fibrotic reaction. However, based on our clinical data, we have demonstrated the great interest of our approach and device. The analysis of the signal stability (based on ECoG signal magnitude (RMS), signal to noise ratio, band power levels of the signal spectrum) was performed on three participants. The signal stability of the 1st participant has highlighted an outstanding signal stability even over a three years implantation period. For the two following participants with a respective follow-up duration of 1.5 year and 6 months, the signal stability follows the same trend.Thanks to this good stability performance, we can foresee uses of the WIMAGINE ECoG recorder during rehabilitation period or for chronic use in the participant's daily life. Note that these implants embedded in the skull thickness can be considered as passive/inert devices once the head-set providing the power thanks to the inductive link at 13.56 MHz. **Outlook**: So far, the WIMAGINE implants coupled proprietary decoding algorithms[1], [2] have demonstrated their interest for long-term recording and/or decoding of the ECoG signals. This system is compliant with the MDR 2017/745 regulation and is easy to implant. So far, we have demonstrated that it can be used in implantable BCI projects to drive external or implantable effectors. **References:** [1] A. Moly *et al,* "An adaptive closed-loop ECoG decoder for long-term and stable bimanual control of an exoskeleton by a tetraplegic", DOI: 10.1088/1741-2552/ac59a0. [2] A. L. Benabid *et al.*, "An exoskeleton controlled by an epidural wireless brain-machine interface in a tetraplegic patient: a proof-of-concept demonstration", DOI: 10.1016/S1474-4422(19)30321-7.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

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Title: Real-time decoding of electrocorticography signals for an adaptive brain-spine interface in a patient with spinal cord injury

Authors: *F. MARTEL[1], H. LORACH[2,3], A. GALVEZ[2,3], V. SPAGNOLO[2,3], N. INTERING[3,4], S. KOMI[2,3], R. DEMESMAEKER[3,4], C. HARTE[3,4], S. KARAKAS[1], F. SAUTER-STARACE[1], O. FAIVRE[1], V. AUBOIROUX[1], S. CHABARDÈS[5], J. BLOCH[2,3,4], G. CHARVET[1], G. COURTINE[2,3], T. AKSENOVA[1];
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Abstract: Introduction: The clinical study entitled: “Brain-controlled Spinal Cord Stimulation in Patients With Spinal Cord Injury” (STIMO-BSI, NCT04632290, clinicaltrials.gov) aims to restore natural motor control of the lower limbs after spinal cord injury and improve neurological recovery with training. Online decoding of epidural electrocorticography (ECoG) recordings is required to control epidural electrical stimulations below the injury to restore walking capabilities. **Methods:** The decoding algorithm is based on the Recursive Exponentially Weighted Markov-Switching multi-Linear Model (REW-MSLM) algorithm developed at CLINATEC [1]. It is composed of two decoding layers. A first dynamic classification layer called gate is able to translate brain signals into movement states probabilities. A second layer consists in multiple multilinear models called experts allowing the control of continuous movements or stimulation amplitudes. The overall output of the decoder is obtained mixing the output of experts according to their state probabilities estimated by the gate model. Each expert and the gate model can be independently and incrementally trained during online control experiments, in parallel of movement predictions. The decoding model is modular and is adapted to the use cases: the number of classification states, experts, and their hierarchical organization are configured according to the use case. **Results:** Models for single joint movements could be calibrated online within a few minutes and provide an accuracy above 90%. The iterative nature of the algorithm enabled constant refining of the models without impairing the training of the participant. The participant used different models over a period exceeding 6 months to control stepping onset, step height, as well as independent joints movements for rehabilitation exercises. Our paradigm allows tasks-specific models to be built, which have been studied to determine the neuronal features of interest depending on the movements attempted, in regards to the spatial, temporal, and frequency modalities. **Conclusion:** The decoder used for the BSI project allowed to distinguish between the hip, knee, and ankle activities (bilateral), trigger stepping events during gait, as well as modulate the amplitude of the steps during walking in order to restore a natural and smooth control of the lower limb movements. The long term use of the models over a six-month training period, provides insights regarding the differentiating features in the ECoG signals. **Reference:** [1] Alexandre Moly et al 2022 *J. Neural Eng.* **19** 026021. doi: 10.1088/1741-2552/ac59a0. PMID: 35234665.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 642.04

Topic: E.05. Brain-Machine Interface

Support: Defitech Foundation
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Pictet Group Charitable Foundation
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Swiss National Science Foundation NCCR Robotics and Project 205563
ERC-2019-PoC BRAINGAIT 875660
EIC-2021-TRANSITIONCHALLANGES-01-01

Title: Motor cortical encoding of lower limb voluntary movements for a brain-spine interface.

Authors: *A. GALVEZ[1,2], V. SPAGNOLO[1,2], N. INTERING[3,2], F. MARTEL[4], S. A. KOMI[1], R. DEMESMAEKER[1,2], C. HARTE[1,2], S. KARAKAS[4], T. ASKSENOVA[4], O. FAIVRE[4], V. AUBOIROUX[4], F. SAUTER-STARACE[4], S. CHABARDÈS[5], G. CHARVET[4], J. BLOCH[1,2,3], G. COURTINE[1,2], H. LORACH[1,2];
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Abstract: Introduction: Voluntary movements require commands from the motor cortex in order to plan a voluntary action, coordinate movement sequences and execute proper adaptive behavior strategies. These motor cortical commands descend to lower level motor hierarchy areas in the brainstem and the spinal cord to produce the intended motor behavior. After a spinal cord injury (SCI) this communication between the brain and the spinal cord is interrupted with devastating impact on motor control and sensory functions. In order to demonstrate the feasibility of prosthetic control from cortical signals, we characterized the brain features related to lower limb motor attempts after SCI.

Methods: In the context of the STIMO-BSI study (NCT04632290) we used a fully wireless implanted epidural electrocorticography technology (WIMAGINE®) to record bilateral activity over the sensory motor area. We studied the encoding of motor behavior which is associated with spatio-temporal spectral changes of neuronal activity across sensorimotor brain areas, specifically patterns of synchronization and desynchronization in different frequency bands.
Results: In static position, we were able to identify the key features to discriminate different joint movements for up to 6 degrees of freedom. We asked the participant to attempt hip flexion, knee extension and ankle dorsiflexion from both sides while sitting. The spatial, spectral and temporal features for lower limb movements were clearly segregated from upper limb motor features. By coupling the decoded motor intentions to epidural spinal cord stimulation, we were able to selectively control those muscle groups. In locomotion tasks, we identified the cortical features related to the different phases of gait with high accuracy enabling a robust control of stepping in a variety of environments and ground conditions. We compared the sensory related activity to the intention related activity and we quantified the variability of the features to the different environmental conditions and task complexity.
Conclusion: We were able to identify the motor and sensory brain features of the lower limbs. By using the brain spine interface, we could isolate the feedback from the external sensory component by epidural spinal stimulation and permit decoding of motor intentions with high accuracy. These results show that the encoding of motor intentions to execute lower limb movements can be extracted even after spinal cord injury and separated from sensory inputs provided by either natural inputs or through spinal cord stimulation. This work supports the feasibility of the Brain-Spine Interface approach to restore voluntary motor control after SCI.

Disclosures: **A. Galvez:** None. **V. Spagnolo:** None. **N. Intering:** None. **F. Martel:** None. **S.A. Komi:** None. **R. Demesmaeker:** None. **C. Harte:** None. **S. Karakas:** None. **T. Asksenova:** None. **O. Faivre:** None. **V. Auboiroux:** None. **F. Sauter-starace:** None. **S. Chabardès:** None. **G. Charvet:** None. **J. Bloch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); holds various patents in relation with the present work, shareholder of ONWARD Medical. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); holds various patents in relation with the present work, shareholder of ONWARD Medical. F.Consulting Fees (e.g., advisory boards; consultant with ONWARD Medical. **H. Lorach:** None.

Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

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Topic: E.05. Brain-Machine Interface

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Swiss National Science Foundation 205563
Swiss National Science Foundation NCCR Robotics
ERC-2019-PoC BRAINGAIT 875660

Title: System integration of a brain spine interface for home use by spinal cord injury patients

Authors: *S. KARAKAS[1], C. HARTE[2,3], S. KOMI[4,2], A. GALVEZ[4,2], F. MARTEL[1], V. SPAGNOLO[4,2], N. INTERING[2,3], R. DEMESMAEKER[2,3], T. AKSENOVA[1], F. SAUTER-STARACE[1], O. FAIVRE[1], V. AUBOIROUX[5], H. LORACH[4,2], S. CHABARDES[5], J. BLOCH[4,2,3], G. CHARVET[1], G. COURTINE[4,2];
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Abstract: Introduction: The objective was to develop and integrate a brain-spine interface (BSI) system on a walker to allow paraplegic patients to use the system independently at home.
Methods: The BSI investigational system integrates 2 main components. The first components is the WIMAGINE-BSI system composed of a set of 2 battery-less brain implants WIMAGINE to record ECoG signals on 64 channels through the duramater, a headset providing power to the implants, the WIMAGINE base station allows ECoG transfer to a laptop with the software chain. The software chain is composed of several software including a brain signal decoder, which interprets the collected ECoG data in order to decode the patient's movement intention in the control of the lower limb. Different decoding scenarios combine the detection of one or more states with or without decoding of the amplitude of movement, depending on the training session. There are two steps in the decoding session, the calibration of the decoding model and the free use of an existing model. For the home application, the patient uses an existing model. The second component is the spinal cord stimulation (STIMO) system, composed of applications (G-Drive+ and NRPA, software), and the Activa RC Implantable Pulse Generator and the MDT Specify 5-6-5 which applying temporal stimulation at different amplitudes in order to elicit the desired movements.
Results: We integrated the system on a walker considering the constraints of safety, power and optimum usability by paraplegic participants. We combined our entire software chain on a laptop and optimized the user interface for independent use by paraplegic participants. We optimized the time required to launch a session, the robustness of the system and the reliability of the models to reduce the need for constant recalibration. The participant was able to launch sessions independently in less than 5 minutes and used the system twice per week and the same decoding model was used for several weeks with stable performance.
Perspectives: This prototype of a mobile brain spine interface system for independent use of participant is a first step towards the development of a fully integrated system (embedded) and autonomous in energy for a daily use at home.

Disclosures: **S. Karakas:** None. **C. Harte:** None. **S. Komi:** None. **A. Galvez:** None. **F. Martel:** None. **V. Spagnolo:** None. **N. Intering:** None. **R. Demesmaeker:** None. **T. Aksenova:** None. **F. Sauter-Starace:** None. **O. Faivre:** None. **V. Auboiroux:** None. **H. Lorach:** None. **S. Chabardes:** None. **J. Bloch:** E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); hold various patents in relation with the present work, shareholder of ONWARD Medical. **G. Charvet:** None. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); hold various patents in relation with the present work, hold various patents in relation with the present work. F. Consulting Fees (e.g., advisory boards); consultant with ONWARD Medical.

Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 642.06

Topic: E.05. Brain-Machine Interface

Support: French Ministry of Health (Grant PHRC-15-15-0124)
Institut Carnot
Fonds de Dotation Clinatec

Title: Proof of concept of an auto-adaptive ECoG-based Brain Machine Interface: toward assistance-free neuroprosthetics at home

Authors: V. ROUANNE[1], F. MARTEL[1], S. KARAKAS[1], T. COSTECALDE[1], F. SAUTER-STARACE[1], S. CHABARDÈS[2], ***G. CHARVET**[1], T. AKSENOVA[1];
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Abstract: Introduction: Motor Brain Machine Interfaces (BMIs) aim at translating brain neural signals into commands to effectors. The ongoing two clinical trials (STIMO-BSI - NCT04632290, and 'BCI&Tetraplegia' - NCT02550522) raise great hopes for SCI patients, effectively assessing the feasibility of chronic Electrocorticography (ECoG)-based motor BMI with the WIMAGINE implants. Brain activity decoding to supply motor functions supposes to calibrate a decoding model. The usability of BMIs will be improved by alleviating the need of constant decoder recalibration in a supervised manner and in well-controlled environments. **Methods**: The auto-adaptive framework, which is intended to calibrate the neuronal activity decoder in adaptive manner in real time during the neuroprosthetics self-directed use is explored. The auto-adaptive BMI (A-BMI) approach adds a supplementary loop with the so called Neural Response decoder, which evaluates the level of coherence between user's intended motions and effector actions, based on neuronal data. The prior predictions of the Motor Control decoder are considered as correct or erroneous according to the Neural Response decoder output. It allows revealing in real time during BMI self-directed use the labels needed to calibrate/update the conventional Motor Control decoder. **Results**: A proof of concept study was undertaken to explore the feasibility of A-BMI. The dataset used in offline simulation of online use consisted of ECoG neural data collected with one subject (NCT02550522)[1]. The subject used motor imagery to control continuous movements of a 2D cursor in 19 sessions spaced over 47 days.

The Motor Control decoder was train from scratch using labels inferred from the output of the Neural Response decoder. We report a cosine similarity between the output of the Motor Control decoder (trained in this way) and the ideal trajectory of 0.159, compared to 0.211 with a supervised training, and -0.023 for a chance level. **Perspectives**: These promising results should first be replicated in the context of the ongoing clinical trials during online use of the BCI. The prospects will be the development of an assistance-free neuroprosthetics for home-use. [1] Benabid, Alim Louis, et al. "An exoskeleton controlled by an epidural wireless brain-machine interface in a tetraplegic patient: a proof-of-concept demonstration." The Lancet Neurology 18.12 (2019): 1112-1122.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

Location: SDCC Halls B-H

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Program #/Poster #: 642.07

Topic: E.05. Brain-Machine Interface

Support: NIH DP5-OD029571
Meta Reality Labs Award #2990450277899571
NCATS TL1TR002540
NSF Award No. GRFP-22336377

Title: Proportional electromyographic control of a bionic arm in participants with chronic hemiparesis, muscle spasticity, and impaired range of motion

Authors: *C. J. THOMSON[1], J. A. GEORGE[2];
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Abstract: The long-term goal of this research is to restore intuitive and proportional motor control to stroke patients with an assistive electromyographic (EMG)-controlled exoskeleton. Stroke is the leading cause of disability in the United States, with 80% of stroke-related disability in the form of hemiparesis. Hemiparesis is the paralysis of one half of the body and can be manifested through spastic overactive muscle activity or flaccid weak muscle activity. Current assistive EMG-controlled exoskeletons do not allow for fine force or position regulation. That is, current control strategies provide only binary, all-or-nothing, control. In this study with hemiparetic stroke patients, we show that a Kalman filter can provide robust proportional control of a bionic arm despite paretic muscle activity. We recruited four participants with spasticity that struggle with hemiparesis (modified Ashworth Scale scores of 2.25 ± 0.96, mean ± standard deviation). To train the Kalman filter, the participants' muscle activity was recorded while they mimicked, or attempted to mimic, preprogrammed movements of a virtual bionic hand. Participants mimicked ten hand grasps and hand openings (finger extensions). To test real-time

proportional control, the participants completed a virtual target-touching task. EMG signal-to-noise-ratio (SNR) and performance on the target-touching task was compared between the participants' healthy and paretic arms. All participants successfully completed the virtual target-touching task with their paretic arm. Overall, EMG SNR and proportional control from the paretic arms were comparable to that from the healthy arms. Median EMG SNR from the paretic arms was 11.7% worse for grasping and 67.4% worse for opening. For the target-touching task, median root mean square error for the paretic arms was 19.5% better for grasping and 169% worse for opening. Similarly, median percent time in target for the paretic arms was 3.6% better for grasping and 45.2% worse for opening. For all three metrics, we found no significant differences between the healthy arms' performance and the paretic arms' performance, although sample size was relatively limited (N = 4). These results show promise in the ability to extract proportional control from spastic EMG to control assistive exoskeleton devices, particularly for hand grasping. Future work will validate these findings with additional stroke patients with varying presentations of hemiparesis through real-time control of a virtual bionic arm and physical exoskeleton.

Disclosures: C.J. Thomson: None. **J.A. George:** None.

Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 642.08

Topic: E.05. Brain-Machine Interface

Support: NCCR Robotics SENSIBLE-EXO

Title: Robotic soft exoskeleton combined with sensory-motor stimulation to enhance hand functions in people with hand impairments

Authors: *A. CIMOLATO[1], J. KENEL[1,2], M. RAZZOLI[1,2], S. BELLOMO[1,2], J. DITTLI[2], R. GASSERT[2,3], O. LAMBERCY[2,3], G. VALLE[1], S. RASPOPOVIC[1];
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Abstract: Around the world 50-60 million people with neurological diseases or trauma live with sensory-motor hand impairments that affect their quality of life. Previous studies in people with Spinal Cord Injury (SCI) or stroke proved the potential of robotic hand exoskeletons as rehabilitative or assistive tools, but also highlighted room for improvement in terms of functional assistance and fine grasp force control.
To this aim, we developed a SensibleExo combining a light and fully portable sensorized hand exoskeleton with sensory-motor electrical stimulation. This neuro-assistive device has the objective to improve the fine motor control in grasping through augmented sensory feedback and neuromuscular stimulation in people with hand impairments.

Our device demonstrated to improve grasping ability and increase the number of objects that users with hand impairments can manipulate during daily living activities. We initially validated our system measuring the increment in grasping force and finger range of motion combining the support of the exoskeleton and neuromuscular stimulation on the hand muscles. Using Transcutaneous Electrical Neural Stimulation (TENS), we enhanced the users' residual sensory feedback providing functionally-relevant sensory information about touch location and intensity during object-hand interaction. We finally assessed the benefit of combining both sensory and motor restoration in terms of the users' motor control while manipulating fragile objects.
The SensibleExo is the first neuro-assistive wearable device able to provide assistance to the sensory-motor control in people with hand impairments providing new opportunities to improve hand functionality in their daily living.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

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Program #/Poster #: 642.09

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS121079

Title: Somatotopically-congruent imagery enhances BCI control in human participants

Authors: *N. G. KUNIGK[1,2,4], B. M. DEKLEVA[1,3,4], A. J. HERRERA[1,2,4], F. LIU[1,3], S. M. CHASE[5,4,6], M. L. BONINGER[1,3,2,7], J. L. COLLINGER[1,3,2,4,7];
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Abstract: Intracortical brain-computer interfaces (BCIs) have the potential to restore motor function and improve the quality of life of many people with paralysis and other motor impairments by providing control of prosthetic limbs and interaction with computers. In human BCI studies, microelectrode arrays are often targeted to the 'arm and hand' area of motor cortex, sometimes guided by presurgical functional imaging. In most cases, new BCI users are able to swiftly attain neural control of computer cursors using imagined reaching movements to generate patterns of neural activity that are decoded as velocity inputs to the cursor. The first two participants in our study (P1 & P2) successfully used reach-related imagery for cursor control and high-degree-of-freedom control of a robotic limb. However, one participant in our study (P3) experienced unusual difficulty in using the BCI for cursor or robotic arm control when using

reach-related imagery despite good signal quality on the majority of channels across microelecrode arrays. In addition, this participant has very high levels of performance when controlling BCI output modulated by imagined movements of individual fingers. Conversely, P2 has achieved less success on BCI tasks involving individual finger control. In order to determine the source of this inconsistency between study participants, we developed an experimental paradigm to study neural activity during movements of joints in the arm and hand. Channels were considered modulated for a specific movement if the firing rate during movement was significantly different from the baseline (rest) period. P2 displayed significantly more elbow- and shoulder-modulated channels across arrays, while P3 displayed almost no elbow- or shoulder-modulated channels in either array. In contrast, P3 displayed significantly more hand- and wrist-modulated channels across both arrays. Based on these results, we re-attempted a 2D center-out cursor task with P3, instructing the participant to imagine moving his wrist to control the cursor rather than imagining reaching movements. Immediately upon attempting control with this new mental imagery, P3 was able to successfully complete over 90% of center-out task trials. Despite recent human studies showing that the 'arm and hand' area of motor cortex is active for movements ranging from the mouth to the feet, we found evidence of somatotopic organization in our multielectrode recordings that influenced BCI control. While additional training and learning may enable successful BCI control for a variety of imagery and decoding strategies, using somatotopically-congruent imagery can provide an immediate performance advantage.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 642.10

Topic: E.05. Brain-Machine Interface

Support: NIH R01NS102259

Title: Non-invasive hands-free control of a robotic arm

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Abstract: Simple tasks such as using a computer, feeding oneself, personal hygiene, and grabbing objects are impossible for high level tetraplegics without assistance. However, most of these individuals retain the ability to voluntarily move their head and can speak. It seems reasonable to hypothesize that signals derived non-invasively from these actions could be used to accurately control movements of a robotic limb or a paralyzed upper limb using functional electrical stimulation. The goal of this project was to evaluate various hands-free methods to control the endpoint of a robotic arm. Able-bodied adults participated in each of four experimental sessions. Subjects used one of four input methods to move the tip of a robotic arm to different targets. In one session, head position/orientation was mapped to the position of the robotic arm, while in a different session, head position/orientation was instead mapped to robot arm velocity. In another session, subjects used discrete voice commands to control movements of the robotic arm. Finally, as a benchmark comparison, the position of the subjects' hand controlled the robotic arm. When using the head or hand, signals from small 6 degree-of-freedom movement sensors attached to those body parts were used as inputs to the robot arm. Once instrumented and having practiced, subjects performed a set of 72 trials, involving 6 reaches to 12 different targets for each session. Physical targets were wooden rings mounted on supports of different heights and designated the 5-cm radius virtual sphere target. For a successful reach, the tip of a pointer attached to the robot arm needed to be situated and held for 1 s within the target. The main dependent measure was movement time (time from exit of the start position to successful capture of the target). There was a significant effect of input modality on movement time ($p < 0.001$, repeated measures ANOVA), with averages (± SD) for hand, head (position), head (velocity), and voice control, 5.7 ± 1.6 s, 6.5 ± 2.4 s, 14.4 ± 4.2 s, and 19.5 ± 4.1 s, respectively. Post-hoc analysis, however, revealed no significant difference ($p = 0.68$) between hand and head position control. This outcome demonstrates the considerable utility of head movements to control robotic arms. Such a non-invasive method could enable individuals with high level paralysis to interact with their environment in complex ways and greatly enhance their independence, health, and sense of well-being.

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Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

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Program #/Poster #: 642.11

Topic: E.05. Brain-Machine Interface

Support: NSF Award No. 1646204
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Title: Motor control policies are conserved from single isotonic to multi-joint movements of the upper limb

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Abstract: It is well known that upper limb movements show characteristic control relationships when performed within certain constraints. For example, the path of the hand for healthy human subjects performing point-to-point reaching movements follows a minimum jerk trajectory (a trajectory that minimizes the third derivative of the hand position), with a bell-shaped velocity profile. Although this control policy has been demonstrated for a variety of multi-joint coordinated movements, it is unknown if this control property is also exhibited at the single joint or muscle level. Demonstrating similar control relationships for the activation of muscles would provide strong evidence for a common control policy throughout the different levels of the motor system, from complex multi-joint limb control to individual muscle compartments or single joints. Here, using ultrasound imaging (sonomyography), we show that the control derived from the activation of forelimb muscles during isotonic movements in a target acquisition task is comparable to performance in a similar arm reaching task. Specifically, the velocity profiles derived from imaging muscle activation patterns followed a similar minimum jerk trajectory shown for point-to-point arm reaching movements, with similar time to target. Analogous to arm reaching, the trajectories based on sonomyography followed a minimum jerk trajectory, resulting in a systematic scaling in peak movement velocity, and delay in time for the peak to occur as the movement distance increased. The peak velocity increased significantly with respect to the movement distance for both control modalities ($p<0.05$). Additionally, the time taken by subjects to acquire the target increased significantly as movement distance increased for both control modalities ($p<0.05$). The position traces were consistent, with very low average standard deviation across trials (1.3% for manipulandum, 4.4% for sonomyography). From the shortest to the longest movement distance the peak velocity increased by a factor of 4.24 for the manipulandum and 4.93 for sonomyography. However, over the same distance range the normalized time to target increased by a factor of 2.46 for the manipulandum and 1.83 for sonomyography. These results demonstrate that motor planning based on muscle activation follows the same movement properties of multi-joint limb movement, suggesting a common control policy despite the execution involving different levels of the motor system.

Disclosures: S. Patwardhan: None. **J. Schofield:** None. **W.M. Joiner:** None. **S. Engdahl:** None. **S. Sikdar:** None.

Poster

642. Motor Control and Neural Prosthetics to Recover Motor Function

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 642.12

Topic: C.04. Movement Disorders other than Parkinson’s Disease

Support: NIH NINDS R15 NS087447-02
NSF 1806056

Title: The effect of afferent feedback on tremor propagation: a modeling study

Authors: *I. SYNDERGAARD[1], D. FREE[2], D. FARINA[3], S. K. CHARLES[1];
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Abstract: Although tremor is the most common movement disorder, how tremor spreads from descending neural drive to tremor at the joints is not fully understood. A thorough understanding of tremor propagation is necessary to determine which muscles contribute most to tremor and should therefore be targeted for treatment. Previous studies have characterized the effects of mechanical coupling (due to musculoskeletal geometry and mechanical impedance) on tremor propagation; our current study aims to characterize the effects of neural coupling due to afferent feedback. We expanded an open-loop model by Corie et al. (2019) to include the effects of homonymous and heteronymous feedback from muscle spindles (type Ia and type II, modeled collectively as proportional-derivative feedback), heteronymous feedback from Golgi Tendon Organs (modeled as force feedback), and propagation delay. The inputs to this closed-loop model were the tremorogenic neural drives from the central nervous system to the 15 major superficial muscles of the upper limb, and the outputs were tremor in the 7 degrees of freedom (DOF) from shoulder to wrist. Thousands of simulations were run using different sets of physiologically plausible feedback gains and delays gathered from the literature to determine the magnitude ratios for each input-output (muscle-DOF) pair. Magnitude ratios were compared between models without any feedback, with GTO force-feedback only, with muscle-spindle proportional-derivative feedback only, and with full feedback. GTO force feedback was found to increase the system's response at tremor frequencies. Furthermore, force feedback was found to decrease the system's response to low-frequency drives typical of voluntary movement, necessitating greater neural drive to complete a task, which may also increase the tremorogenic portion of the neural drive. However, force feedback did not significantly alter the pattern of tremor propagation, i.e. which muscles contributed most to tremor in which DOF. Muscle-spindle proportional-derivative feedback was found to have only a modest influence on tremor and therefore did not substantially alter patterns of tremor propagation either. These results were robust to physiologically plausible changes in feedback and delay parameters. According to our simulations, peripheral neuromuscular feedback moderately exacerbates tremor, but it is not a major contributor to the propagation of tremor. We conclude that mechanical coupling, which does significantly spread tremor (Corie et al., 2019), is the major contributor to tremor propagation.

Disclosures: I. Syndergaard: None. **D. Free:** None. **D. Farina:** None. **S.K. Charles:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.01

Topic: E.06. Posture and Gait

Support: BSF grant 2019222

Title: Implementing self-selected gait speed in virtual reality for research- fully immersive head mounted device versus semi-immersive large screen-based device

Authors: M. PLOTNIK[1,2,3], Y. BAHAT[1], N. GALOR[1], S. KIMEL-NAOR[1], M. WILF[1]; [1]Ctr. of Advanced Technologies in Rehabil., Sheba Med. Ctr., Ramat Gan, Israel; [2]Fac. of Med., [3]Sagol Sch. of Neurosci., Tel-Aviv Univ., Tel Aviv, Israel

Abstract: Background: The use of large-scale virtual reality (VR) systems in combination with self-paced treadmill (SP-TM) enables gait research that simulates everyday conditions. However, such large-scale systems are relatively expensive and thus, rare. The objective of the present study was to validate a newly developed method that enables walking on SP-TM while using Head Mount Display (HMD). **Methods:** Fourteen young healthy participants (mean age [±SD] 32.4 ± 6.3 years; 8 females) walked for 303 seconds on a SP-TM under three different conditions, randomly presented: (1) Without any visual flow; (2) with a visual flow synchronized with their walking speed presented on large screen (51’’); (3) wearing HMD synchronized with their walking speed. (HTC-VIVE, Taiwan). Likert questionnaires were introduced to assess the level of subjective reassurance and comfort while walking in the different trials (seven questions, score = 35- Maximal reassurance, Score=5- minimal reassurance). Gait speed profiles (V) were fitted to the function of where X is the distance passed and *a* is the estimation of the asymptotic steady state velocity value. From the point where the participant reached 95% of the estimated walking speed, we also calculated the mean value of gait speed (GS) and the coefficient of variation (GS-CV= 100*SD/GS). **Results:** Thirteen participants completed all three experimental trials, and their subjective evaluation of the HMD-SP TM walking was comparable to the large screen-based VR system SP TM walking (24.7±5.7 vs. 27.9 ± 3.8, p=0.078). GS was significantly lower in the HMD-SP TM condition as compared to the large screen condition (1.02 ± 0.14 m/s vs., 1.23 ± 0.22 m/s, respectively, p=0.022) and significantly lower than the no visual flow condition (1.25±0.23 m/s, p=0.019). GS did not differ significantly between the no visual flow and the large screen walking conditions (p=0.49). GS-CV was significantly higher in the HMD-SP TM condition as compared to the large screen condition (14.4 ± 12.3 % vs. 4.5 ± 1.9%, respectively, p=0.010) and significantly higher than the no visual flow condition (5,2 ±1.5 %, p=0.037). GS-CV did not differ significantly between the no visual flow and the large screen walking conditions (p=0.20). **Conclusions:** This study suggests that walking in self-pace mode while wearing HMD is feasible in terms of the user comfort and acceptance. Gait performances as reflected by GS and GS-CV fall short from meeting the values of walking in the large screen and the 'no visual flow' conditions. Future studies should address whether this problem can be overcome by training before the introduction of rehabilitation treatments on such platform.

Disclosures: M. Plotnik: None. **Y. Bahat:** None. **N. Galor:** None. **S. Kimel-Naor:** None. **M. Wilf:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.02

Topic: E.06. Posture and Gait

Support: NIH P20-GM109040
VA Career Development Award-2 RR&D N0787-W
American Society of Biomechanics Graduate Student Grant-in-Aid

Title: Investigating the intermuscular coherences of the ankle plantarflexors during treadmill walking in individuals post-stroke

Authors: *C. C. CHARALAMBOUS[1], M. G. BOWDEN[2], J. LIANG[3], S. A. KAUTZ[4], A. HADJIPAPAS[5];
[1]Univ. of Nicosia, Univevrsity of Nicosia Med. Sch., Nicosia, Cyprus; [2]Hlth. Sci. and Res., Ralph H. Johnson VA Med. Center/Medical Univ. of South Carolina, Charleston, SC; [3]Physical Therapy, Univ. of Nevada, Las Vegas, Las Vegas, NV; [4]Hlth. Sci. and Res., Med. Univ. of South Carolina - Col. of Hlth. Professions, Charleston, SC; [5]Univ. of Nicosia Med. Sch., Nicosia, Cyprus

Abstract: After stroke, walking capacity may be limited due to degraded neural drive from the corticospinal tract (CST) and corticoreticulospinal tract (CReST). Intermuscular coherence (IMC) represents a measure of the common neural drive to muscle(s) and exhibits frequency specificity. The drive from CReST putatively results in alpha, whereas that from CST results in beta and low-gamma-band coherence. Previous work showed that coherences in beta band are predominantly present in the plantarflexors (PF: lateral and medial gastrocnemius; LG and MG) and dorsiflexors (DF) during stance and swing phase, respectively, and may be degraded after stroke. DF have been investigated more thoroughly, as these may receive more CST-derived input. Evidence concerning PF in stroke remains very limited. Here, we aim to investigate the interlimb differences in LG-MG IMC and its relationship to propulsive impulse (PI: a LG-MG specific mechanical output) in individuals post-stroke during walking. Fourteen participants (6 females; mean±SD; age: 62±13 years; time post-stroke: 35±27 months; paretic side: 11 right; Fugl-Meyer lower extremity: 26±3) walked 3×30 sec trials on an instrumented treadmill with self-selected walking speed (0.51±0.22 m/sec). Surface LG and MG EMG and ground reaction forces (GRF) were collected and sampled at 2kHz. The LG-MG IMC was estimated (IMC_{Raw}) based on a window comprising the phase in stance in which PF are most active (single leg stance and second double limb support). A z-transformed estimate of coherence mitigating unequal degrees of freedom across patients was also obtained (IMC_Z). The area under the curve from IMC_{Raw} and IMC_Z was estimated and served as the measure for statistical analyses. PI was calculated using anteroposterior GRF during the second half of stance. We statistically compared the inter-limb differences in IMC (Wilcoxon Rank Sum Test) and correlations between IMC and PI (Spearman Rho). For estimating correlations only statistically-significant unilateral IMC estimates were used. Preliminary results indicate that, compared to the non-paretic side, only the paretic beta IMC_{Raw} was significantly (p=0.0258) different (non-paretic > paretic). Unilaterally, only alpha coherence was significant. On the paretic leg, alpha IMC was correlated with PI

(IMC_{Raw}: r_s=0.7, p=0.007; IMC_Z: r_s=0.7, p=0.006). No significant correlations were found on the non-paretic leg. Our results agree with previous work (overground walking) showing a degradation of the paretic LG-MG IMC after stroke and further, suggest a strong relationship between the alpha coherence (a potential proxy of CReST) and LG-MG specific mechanical output on the paretic side.

Disclosures: C.C. Charalambous: None. **M.G. Bowden:** None. **J. Liang:** None. **S.A. Kautz:** None. **A. Hadjipapas:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.03

Topic: E.06. Posture and Gait

Title: Acute neural adaptations to regulated and unregulated low-load blood flow occlusion in quadriceps muscle.

Authors: *H. S. BAWEJA[1], M. D. ROSENTHAL[2];
[1]Physical Therapy, San Diego State Univ., San Diego, CA; [2]Physical Therapy, Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: We have previously reported differentiating blood flow occlusion devices based upon objective measures such as influence on average and peak muscle activation and perceptual measures (pain and exertion). However, acute temporal adaptions in muscle activation to regulated and standardized blood flow occlusion remain unknown. Comparing various aspects of muscle activation patterns during exercise with different BFRT devices, will provide insight into which device may be most effective for specific rehabilitation needs. PURPOSE: The aim of our study was to determine the differences in acute muscle activation between two BFRT devices and traditional high-load training. SUBJECTS: 34 healthy subjects (18 male, 16 female). METHODS: Muscle activation was recorded via sEMG during knee extension under external load for each condition and normalized to each subjects' maximum voluntary isometric contraction recorded at the beginning of the same session. Each subject performed all three training conditions, and session order was randomized. In addition, subject reported perceived exertion were recorded during each condition. Muscle activation was quantified as peak activation (%MVIC), time-to-peak activation, and time of sustained activation greater than 30% MVIC over the contraction across all test conditions. RESULTS: Our main findings were - a) time-to-peak activation was fastest with HL training, but similar across BFRT, and reduced with standardized BFRT but not regulated BFRT or high-load exercise; b) the amount of time subjects maintained greater than 30% muscle activation across conditions was greatest in HL exercise when compared with BFRT, and c) females maintained greater than 30% muscle activation across all conditions for ~8% longer when compared with males across all exercise conditions. This difference was exacerbated with regulated BFRT. We've also previously shown that pain

(NPRS) was significantly higher in BFR conditions when compared with un-occluded high-load training especially with occlusion is regulated. CONCLUSIONS: Acute temporal neural adaptations vary with low-load exercise under standardized and regulated BFR. Women are more resilient to LL-BFRT. CLINICAL RELEVANCE: The type of blood flow occlusion needed to increase time to peak muscle activation and sustain sub-maximal contractions over 30% MVIC are different. This would be beneficial for targeted neuromuscular training in rehabilitation settings.

Disclosures: H.S. Baweja: None. **M.D. Rosenthal:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.04

Topic: E.06. Posture and Gait

Support: Natural Sciences and Engineering Research Council of Canada

Title: Using inertial sensors to assess human movements; the potential challenges and rewards

Authors: *C. DUVAL;
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Abstract: Background: In recent years, numerous efforts have been made to explore the possibility of using inertial sensors to capture human movement. The advantages are numerous, beginning with the fact that contrary to optical movement capture systems, they are impervious to line-of-sight issues, and they are relatively easy and inexpensive to use. However, there are still many challenges facing those who intend to use inertial motion capture system to assess the quantity and quality of human mobility in their natural living environment. In previous experiments, we highlighted how inertial motion units (IMUs) were sensitive to the velocity of movement, as well as recording time. In these studies, IMUs were attached to a gimbal table tumbling at different velocity, for different time lengths. The orientation data obtained by the IMUs was compared to that of an optical system. Furthermore, we also assessed IMU performance against an optical system in a magnetically clean and dirty environments during human walking. Results revealed higher velocity was associated with increased errors, as well as being less accurate during recordings lasting more than two minutes. Furthermore, magnetically dirty environments greatly affected the accuracy of IMUs. **Objectives:** (1) highlight the issues related to the use of inertial sensor units (IMU) to capture human movement in natural living environments, and (2) provide a road map for future research with specific targets aimed at improving IMUs' ecological mobility assessment. **Methods:** in this exploratory study we used previous research by our groups using IMUs to identify specific key components of mobility that must be addressed to better quantify and qualify different aspects of mobility in the natural living environment. Then, we built a schematic hierarchical model of interaction between these key

components. **Results:** we present a step-by-step schematic model that includes movement components such as activity detection (initiation and transition between tasks), activity performance, and mobility issues that may affect this performance. In addition, we also propose a signal-to-noise ratio model, putting the eventual detection of mobility impairments in the context of clinical relevance. For each, we present experiments that put forth the use of IMUs to capture those mobility components efficiently. **Conclusion:** to achieve a self-sustained mobility assessment system using IMUs, several issues of accuracy still must be addressed, and most importantly, different aspects of human movement still need to be integrated into a coherent analytical methodology.

Disclosures: C. Duval: None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.05

Topic: E.06. Posture and Gait

Support: NSF DBI 2015317

Title: Characterizing and modeling non-muscular odontophore materials in the Aplysia californica feeding apparatus

Authors: *K. Y. GLADSON, V. A. WEBSTER-WOOD;
Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Understanding the mechanics of animal behavior is essential for understanding how nervous systems control complex behavior [1]. The feeding mechanisms of the sea slug *Aplysia californica* have served as a model for neural control and recent models have integrated both neural control and biomechanics to create behaviorally flexible models of multifunctional *Aplysia* feeding behavior [2]. While multiple aspects of the feeding apparatus muscles and muscular control have been studied, the non-muscular tissues have not previously been characterized. As a result, previous neural control models have not been able to incorporate soft material deformation models for the odontophore, the food grasper within the *Aplysia* feeding apparatus. Accurate models of odontophore deformation are important because the shape of the odontophore can affect the mechanical advantage of muscle forces, altering the odontophore position, which is an important output and potential feedback point for the neural control of feeding. In this work, the radular surface and radular sac, non-muscular tissues in the odontophore, were characterized and modeled for use in finite element analysis of odontophore deflection under load. Samples were taken from four adult *Aplysia* 100-200g and were tested on the day of sample collection. Radular surface and radular sac samples were tested in tension and compression, respectively, at a speed of 5μm per second while submerged in a room temperature artificial saltwater bath, using an MTS Criterion Universal Test System. The mechanical test data

was fit to three material models, a two-term Ogden model, a Neo-Hookean model, and a Hookean model. Quantitative analysis of the radular surface and radular sac mechanical tests showed that the two-term Ogden model had the highest r-squared value, exceeding 0.99 for both the radular surface and the radular sac samples, indicating that these materials deform non-linearly, similar to mammalian soft biological tissues. While the small sample size limits this study, the current data was used to determine material model parameters for a finite element analysis model of odontophore deformation under simplified loading conditions. As a result, this work serves as a pilot study for quantifying previously unreported aspects of odontophore deformation during *Aplysia* feeding for future integration into existing neuromechanical models of *Aplysia* feeding behavior. [1] Chiel HJ, Beer RD. Trends Neurosci. 1997 Dec;20(12):553-7. [2] Webster-Wood, V.A., Gill, J.P., Thomas, P.J. et al. Biol Cybern 114, 557-588 (2020).
2

Disclosures: K.Y. Gladson: None. **V.A. Webster-Wood:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.06

Topic: E.06. Posture and Gait

Support: NSERC

Title: The effect of muscle fatigue on regional activation in the ankle plantarflexors during postural pertubations

Authors: N. GRZYWNOWICZ[1], T. D. IVANOVA[2], *S. GARLAND[2];
[1]Kinesiology, [2]Fac. of Hlth. Sci., Western Univ., London, ON, Canada

Abstract: Introduction: The central nervous system maintains standing balance by activating motor units in the ankle plantarflexor muscles: soleus, medial and lateral gastrocnemius (SOL, MG & LG). Regional modulation of the muscle activity was observed during external perturbations that challenge standing balance in different directions. The purpose of this study was to determine the effect of muscle fatigue in MG on the regional modulation of the activity of SOL, MG & LG during external perturbations. **Methods:** Participants performed a balance test before and after a fatigue protocol by standing on a force platform on their right leg and were pulled in three different directions (0°, 60° left and 60° right) by a load weighing 1% of their body mass. The barycenter and amplitude of muscle activation from three 64-channel high density surface (HDs)EMG grids was calculated from each muscle for each direction of balance perturbation. The fatigue protocol consisted of 20Hz ten-pulse trains delivered once every second for 6 minutes to the MG motor point. The current intensity was adjusted to produce 20% of the participant's maximal voluntary contraction. A supramaximal 100μs pulse to the tibial nerve elicited M-waves and twitches. The amplitude of M-waves and the twitch forces were

monitored during the protocol for the progression of fatigue and to ensure that fatigue was isolated to the MG. The fatigue protocol ended when the force produced by the fatiguing trains decreased to 50% of its initial value. **Results:** There was a significant decrease in the MG M-wave amplitude (p=0.019) and in the force produced by the fatiguing trains (p=0.008) after fatigue. No change in the LG or SOL M-waves was observed. The pre-fatigue balance testing revealed that when perturbed to the left, the barycenter of MG, LG & SOL shifted towards the right. When perturbed to the right, the barycenter of the MG, LG & SOL shifted towards the left. After the fatigue protocol, the barycenter of the LG & SOL followed the same pattern of shifting as in pre-fatigue, but the MG did not. **Conclusions:** The results from the fatigue protocol suggest that low frequency stimulation delivered to the MG motor point produces a marked decrease in force isolated to the MG. In the non-fatigued state, the activity of the MG, LG & SOL was regionally modulated in response to external perturbations in a direction-specific manner. However, post-fatigue, this direction-specific regional modulation of muscle activity was maintained only in the non-fatigued LG & SOL, but not the fatigued MG.

Disclosures: **N. Grzywnowicz:** None. **T.D. Ivanova:** None. **S. Garland:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.07

Topic: E.06. Posture and Gait

Support: NIH R01-HD091184
NIH KL2TR001854

Title: Guided exploration of energetic cost during split belt walking influences locomotor adaptation

Authors: *N. SANCHEZ[1], J. M. DONELAN[2], J. M. FINLEY[3];
[1]Chapman Univ., Irvine, CA; [2]Dept of Biomed. Physiol. & Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada; [3]USC, Pasadena, CA

Abstract: Background: Adaptation of walking can be studied using a split belt treadmill. This form of adaptation can be explained as a process by which people learn to take advantage of mechanical work from the treadmill to reduce the energy cost of walking: by stepping further forward on the fast belt people increase the positive work performed by the treadmill and reduce the positive work performed by the legs. However, the underlying algorithm that people use to find and exploit less costly walking patterns remains unknown. Reinforcement learning is one means by which people may learn the relationship between actions such as foot placement location and their corresponding value (e.g., energetic cost).
Objectives: to determine whether experience with gait patterns of varying energy cost impacts walking adaptation. If people learn to modify their gait through reinforcement, experience of less

costly patterns should serve as a stimulus that helps the nervous system update its prediction of the pattern most likely to minimize energy cost, while preventing people from discovering a less energetically costly gait should hinder adaptation.
Methods: we used visual feedback targets to guide limb placement during split-belt walking: walking with longer steps with the leg on the fast belt (LF) which requires low mechanical work, or walking with longer steps with the leg on the slow belt (LS) which requires high mechanical work. Participants used the targets for five minutes, and then continued to walk without the targets for an additional 10 minutes (NoFBK). 12 participants were assigned to each condition. We compared walking patterns to 15 participants during unguided split-belt adaptation (UG). We used linear models to assess the relationship between limb placement and work by the legs.
Results: We observed differences in limb placement consistent with the targets (LF: 365 +/- 56 mm, UG: 286 +/- 35 mm, $p<0.001$ and LS: 194 +/- 32 mm, UG: 160 +/- 37 mm, $p<0.001$). The positive work performed by the legs during LF and LS was predictive of the work performed early in NoFBK ($R=0.83$, $p<0.0001$) and at the end of the NoFBK period ($R=0.60$, $p=0.0002$); not all participants reduced work, but those that did maintained this low cost strategy during NoFBK.
Conclusions: Our results are consistent with the hypothesis that using visual feedback to guide individuals toward a less energetically costly gait reinforces the use of a less costly gait pattern when feedback is removed. However, providing guidance of kinematic strategies was not sufficient to drive all participants to reduce positive work. Future work should understand factors that explain individual differences in the ability to reduce energetic cost.

Disclosures: **N. Sanchez:** None. **J.M. Donelan:** None. **J.M. Finley:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.08

Topic: E.06. Posture and Gait

Title: Examining the relationship between kinematic patterns and time of typing characterized by physical and environmental restraint conditions

Authors: *T. ITO, M. YOSHIDA, T. KOKUBUN;
Grad. Sch. of Saitama Prefectural Univ., Koshigaya, Japan

Abstract: [Title]Examining the relationship between kinematic patterns and time of typing characterized by physical and environmental restraint conditions[Keyword]Typing, Kinematics, Wrist and Finger Movement 1. Motivation/problem statementThe central nervous system constraints joint degrees of freedom to simplify control to perform accurately. Previous studies have revealed the movement patterns of proximal and distal joints that contribute to the accurate movement (Bizzi+ 1984, Hore et al. 2005, Furuya and Kinoshita 2008). Although the types of wrist and finger movement patterns during typing have been shown (Baker et al. 2007, Feit et al.

2017), the relationship between keystroke time and kinematic patterns has not been described. Keyboard typing is a reaching movement in which keystrokes are continuously entered. The starting and ending points are determined by which key is entered. Therefore, the regularity of controlling the multi-joint movement of typing may differ from that of previous studies. This study aims to clarify the movement patterns that contribute to keystorke time. 2. MethodsSubjects were 10 healthy adults. They provided written informed consent, following a detailed explanation of the study's purpose and risks according to the Declaration of Helsinki. We used a 3D motion capture system (Vicon) and a QWERTY keyboard (Microsoft) to obtain the position of the markers attached to the right hand. The task was 5,000 Japanese characters. The analysis interval consisted of inputting eight trials of the frequently occurring words "ryouhou" in the sentence. Inter-key interval time and changes in the position of the right metacarpophalangeal (MP) and proximal interphalangeal (PIP) joint markers were calculated. We used four of the eight trials with no errors in all subjects, except for two subjects whose number of input errors exceeded the mean +2SD in all subjects. The Ethical Review Committee approved this study. 3. ResultsThe inter-key interval time for each trial converged within the mean ± 2SD. Three subjects showed a more significant change in the PIP joint marker position relative to the MP joint marker. The others showed no difference between the MP joint and PIP joint marker positions. 4. Conclusion/implicationsContext and input error didn’t disturb the time of keystokes. Typists select a pattern of predominant selection of wrist and finger joint movement or synchronization of their movement.These results showed that differences in movement patterns might not affect the temporal consistency of typing. Ignoring body variables involved in movement patterns and changing them may not be appropriate as typing learning.

Disclosures: **T. Ito:** None. **M. Yoshida:** None. **T. Kokubun:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.09

Topic: E.06. Posture and Gait

Title: Simulated gravitational loading alters lower extremity kinematics and electromyographic variables during walking

Authors: *C. A. MALAYA[1], A. RIAZ[2], S. CHANDRASEKARAN[2], Z. MOHIUDDIN[2], C. S. LAYNE[2];
[1]Hlth. and Human Performance, Univ. of Houston, Houston, TX; [2]Univ. Houston, Univ. Houston, Houston, TX

Abstract: Background: Gravity is a primary factor in human gait and movement. It is unknown how quickly the body adapts to new gravitational environments and if these adaptations are robust at certain gravitational levels. This project examined kinematic and electromyographic adaptations to different levels of gravitational load. Better understanding of the effects of specific

levels of loading will aid future rehabilitation techniques, as well as improve our general understanding of how gravity influences human locomotive adaptations.
Objective: To explore the effects of simulated gravitational loading on kinematics and muscle activity during walking.
Methods: 15 healthy adult participants walked in a treadmill-based loading system at a comfortable, self-selected speed. Participants experienced loading levels of 100%, 110%, 120% and 130% of their body weight, for 1-minute each, without the addition of external mass. There was no delay between conditions. Inertial measurement units and surface electromyographic sensors captured joint angles (hip, knee, ankle) and muscle activity (rectus femoris, biceps femoris, medial gastrocnemius, anterior tibialis) of the lower extremities, respectively. Mean and difference waveforms were calculated and subsequently compared using statistical parametric mapping. Phase diagrams, phase diagram areas, root mean square and integrated areas were calculated for each joint and muscle at each level of loading.
Results: Testing revealed differences between conditions and difference waveforms for the hip and ankle, as well as the rectus femoris, biceps femoris and medial gastrocnemius. The anterior tibialis and knee were unaffected. Phase diagram areas for all joints reduced as loading was increased. Similarly, muscle activity - as quantified with root mean square and integrated area calculations - appeared to change differently depending on the muscle, as well as the loading condition.
Conclusions: Greater gravitational loading appears to primarily influences hip kinematics and can influence the coordinative structure of hip movement. The rate of change in muscle activity and joint angles between levels of load are non-linear. This is further supported by phase diagrams for each joint that show non-linear differences in area - corresponding to the statistically significant findings in the statistical parametric mapping outcomes- as the level of loading changes.

Disclosures: C.A. Malaya: None. **A. Riaz:** None. **S. Chandrasekaran:** None. **Z. Mohiuddin:** None. **C.S. Layne:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.10

Topic: E.06. Posture and Gait

Title: Characterizing Muscle synergies for Reactive balance control in post-stroke hemiparetic gait

Authors: *H. KIM, Y.-C. CHUNG, L. ANDERSON, S. SOEDIRDJO, Y. DHAHER;
Univ. of Texas, Southwestern Med. Ctr., Dallas, TX

Abstract: Introduction: Examinations on stroke patients (Zehr et al, 2012) suggested that impaired interlimb reflexes may contribute to post-stroke lower-limb motor discoordination

(Hurteau et al, 2018) Specifically, our earlier examination of stroke indicated that attenuated interlimb reflexes may be contributing to the impaired neuromuscular responses in reactive balance control during gait (Sharafi et al, 2016). Previous studies on reactive balance control during walking have independently investigated the activities of each muscle (Chvatal et al 2011). However, little is known about the post-stroke modular neuromuscular control through muscle synergies in reactive balance control during walking. Accordingly, this study aimed to characterize post-stroke lower-limb muscle synergies for reactive balance control in response to a swing phase perturbation. **Method:** 5 stroke participants received a swing phase perturbation by interrupting the foot via a retractable cable attached to the foot. To minimize anticipation of the trip, 15 perturbations were applied to both feet at random order and intervals, at least 30 seconds apart. Surface EMG was recorded bilaterally from the soleus, medial gastrocnemius, tibialis anterior, biceps femoris, vastus medialis, rectus femoris, adductor magnus, and gluteus medius. EMG responses in the impaired and unimpaired limb during walking (at least 3 gait cycles) and perturbed (up to 300 ms post perturbation) conditions were filtered and quantified for the synergy analysis by principal component analysis. **Results:** Across subjects, three muscle synergies during walking and four synergies in perturbation-induced responses could explain 90% variances in both impaired and unimpaired limbs. In the unimpaired leg, 2 out of 3 synergies during walking showed strong correlations to the synergies identified during perturbation (r > 0.85). While in the impaired leg, 2 out of 3 synergies during walking were considered to be similar (r > 0.623). **Discussion:** Our result indicated that more muscle synergies identified in perturbation induced responses compared to the synergies identified in walking even though walking generally requires a greater number of synergies. Furthermore, our results suggest that stroke patients may rely on a common set of muscle synergies both in locomotion and reactive balance control. The existence of a common set of muscle synergies was previously reported in healthy participants (Chvatal et al 2013). However, it remains to be seen whether synergies reported in healthy control are similar to synergies reported in post-stroke.

Disclosures: H. Kim: None. **Y. Chung:** None. **L. Anderson:** None. **S. Soedirdjo:** None. **Y. Dhaher:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.11

Topic: E.06. Posture and Gait

Support: ERC Salamandra project number 951477

Title: A biologically-realistic neuromechanical model of the salamander, a vertebrate animal model that walks, trots and swims in changing environments

Authors: *A. FERRARIO[1], J. ARREGUIT[1], A. PAZZAGLIA[1], A. SIMON[2], D. RYCZKO[3], A. J. IJSPEERT[1];

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Abstract: Salamanders dynamically adapt their locomotor behaviours in response to changes in the environment. This adaptive control is modulated by the level of descending drive from the reticulospinal neurons (RSD), a highly conserved structure across vertebrates. We hypothesise that at low drives salamanders typically perform a slow lateral or diagonal walk, at intermediate drives they trot at higher speeds, and at even higher drives they swim with frequencies higher than those during trot. Similar transitions have been confirmed experimentally in cats, mice and salamanders. Previous neuromechanical models of the salamander locomotion could reproduce the transition between trotting and swimming as a function of the RSD, but lacked a mechanistic explanation of these transitions based on physiological properties of the neural circuit.
Here we propose a biologically realistic firing rate model for interconnected limb and spinal neural circuits that reproduces walk, trot, swimming and their transitions as a function of the RSD. The various locomotor rhythms are driven by a mechanism of escape from inhibition and slow firing rate adaptation (fatigue), as in previous spiking models of the tadpole, lamprey and salamander spinal cords. The model suggests (1) a weighted inhibition between all limb centres is necessary to achieve an appropriate lateral sequence and diagonal walks, (2) the existence of excitatory interneurons projecting diagonally across the limbs to ensure trot, and (3) the role of ascending/descending pathways in shaping the activity propagation during swimming.
The neural network was paired with a biomechanical simulator of the salamander musculoskeletal system interacting with water and ground. This allowed testing the ability of the model to walk/trot on ground, and swim in water. Furthermore we use this biomechanical model to explore the role of exteroceptive sensory feedback loops (i.e. hydrodynamic and contact forces) in terms of locomotion performance. Unlike previous simplistic models, where the switch from trot to swimming was hand tuned artificially, here this switch is achieved dynamically due to a network transition from an oscillatory trotting regime to a steady state flexor-dominated activity at high drives. At low drives, the network exhibits a steady state extensor-dominated activity, resembling the quadruped position of the limbs during the stance phase. This work will provide the working ground for developing a spiking neural network of the salamander locomotion in closed loop.

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Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.12

Topic: E.06. Posture and Gait

Title: Automatic analysis of locomotion performance score on an open-source treadmill for rats

Authors: C. MÉNDEZ-VALLE[1], M. GAMIÑO-SÁNCHEZ[1], F. CARRILLO-BALLESTEROS[2], J. REGLA-NAVA[1], F. CASILLAS-MUÑOZ[2], D. MARTÍNEZ-FERNÁNDEZ[2], S. LUQUIN[3], D. FERNÁNDEZ-QUEZADA[3];
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Abstract: Motor deficits can significantly affect the completion of daily life activities and harm quality of life. In research laboratories, rats are habitual models used for studies on locomotor activities; however, manual quantification of motor behavior represents a serious challenge since locomotion performance requires the coordination of the CNS regulating extremities movement, balance, postural control, speed, cadence, and other systems involved. Also, some motor deficits may not be as easily detected in rodents as they are in humans, quadrupeds can shift their weight to their forelimbs in the case of a hindlimb injury, where bipeds can not. Therefore, the use of an open-souse analysis program of locomotion performance can be helpful to correlate physiological or metabolic parameters and motor deficits. This research aimed to develop an open-source laboratory treadmill to investigate the impacts of locomotion on neuromuscular and musculoskeletal deficits in small animal models. We designed, built, and calibrated a treadmill that allows the user to program different protocols in terms of speed, time and elevation, the interface includes add-ons, which are a gait analysis app to evaluate the physical performance of each of the test subjects. The score criterion is based on the recording registry of male Wistar rats running for 8 weeks. We found significant differences throughout a training program, providing evidence of this performance score to reflect the individual evolution in motor capacity.

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Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.13

Topic: E.06. Posture and Gait

Support: Deutsche Forschungsgemeinschaft, Research Fellowships Program, Grant SA 3695/1-1
Canadian Institutes of Health Research Grant 162357

Title: Supraspinal integration of proprioception is crucial for locomotor robustness

Authors: *A. SANTUZ[1,2], O. D. LAFLAMME[1], T. AKAY[1];
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Abstract: Vertebrate locomotion in the wild often happens in complex environments. The generation of rhythmic locomotor patterns is in part achieved by spinal neuronal networks called central pattern generators. Yet, when environmental factors challenge locomotion, spinal circuits are not sufficient to ensure locomotor robustness, which is the ability to cope with perturbations. Proprioceptors are sensory organs found mostly in muscles (muscle spindles) and tendons (Golgi tendon organs) and they communicate the position of body parts to the central nervous system. Their signals are conveyed to the brain through the dorsal column of the spinal cord and the spinocerebellar pathways, but the importance for locomotion of their supraspinal integration is not clear. We used a combination of mouse genetics, spinal lesion models, *in vivo* electrophysiology and biomechanics to examine the role of supraspinal integration of proprioception during perturbed locomotion. We hypothesized that not only spinal but also supraspinal processing of proprioception must be involved in the tuning of motor output when locomotion is challenged by perturbations. Wild-type mice (C57BL/6, n = 5) modulated the kinematics (increased variability) and muscle activation patterns (increased duration and decreased complexity or accuracy) of the hindlimb when perturbations were randomly administered in the form of sudden mediolateral displacements or accelerations of the treadmill's belt. In muscle spindle-deficient mice ($Egr3^{-/-}$, n = 5) the locomotor patterns were similar to those encountered in the wild type during perturbed walking, independent on the presence of perturbations. The acute and systemic ablation of both muscle spindles and Golgi tendon organs (PV^{Cre}::$Avil^{iDTR}$, n = 5) had similar, but more pronounced effects. When we surgically lesioned the dorsal column in wild-type animals (n = 5), mice stopped responding to perturbations. Contrary to the animals where proprioceptors were ablated, the locomotor output was undiscernible from that of pre-lesion, unperturbed walking, independent on the presence of perturbations. We showed that animals lacking proprioceptors locomoted in a constantly perturbed state, independent on the administration of mechanical perturbations. This was not the case for wild-type mice, in which we surgically interrupted the flow of proprioceptive feedback to the brain through the dorsal column. After lesion, the animals behaved similarly to unperturbed, pre-lesion walking in both the absence and presence of external perturbations. Our results support a crucial role of the brain in responding to external perturbations and ensuring robust locomotion.

Disclosures: **A. Santuz:** None. **O. D. Laflamme:** None. **T. Akay:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

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Program #/Poster #: 643.14

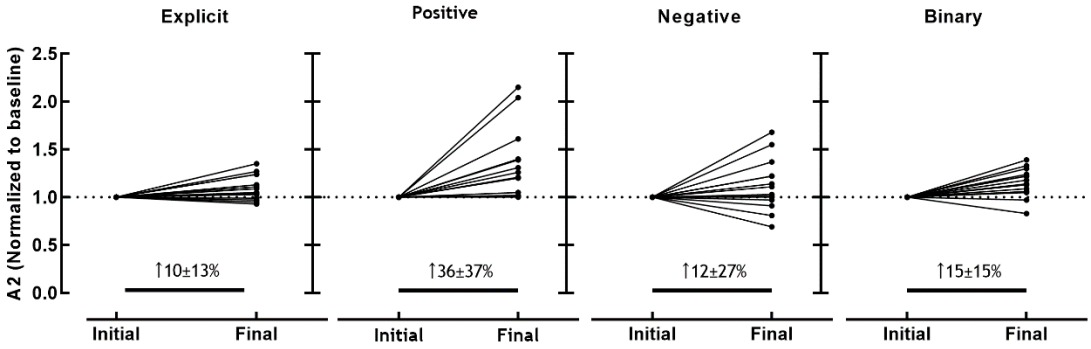
Topic: E.06. Posture and Gait

Support: NSF grant #1935501
VA 1IK6 RX003543
UC Davis School of Medicine

Title: Rewarding performance in a kinetics-based learning task facilitates locomotor learning

Authors: *D. R. YOUNG, T. E. MCGUIRK, E. S. PERRY, W. M. JOINER, C. PATTEN; Univ. of California, Davis, Davis, CA

Abstract: Introduction. Most stroke survivors exhibit gait impairments, reducing community participation. Reward-based motor learning may increase motivation and dopamine-dependent neuroplasticity compared to traditional methods, which produce inconsistent results. Peak plantarflexion power during late stance (A2) contributes most of the anterior propulsion during walking and is commonly impaired after stroke. Here we employed A2-based biofeedback during a learning intervention. Methods. Fifty-six (F=38, 22±3.4y, 1.68±0.09cm, 68.01±15.43Kg) healthy individuals were grouped into one of four feedback conditions: Explicit, Positive, Negative, and Binary. Participants received real-time feedback throughout three 15-minute bouts of walking. Each bout was preceded by a baseline trial and followed by a 10-minute washout. Target leg A2 was calculated and displayed upon the next ipsilateral heel strike. Feedback mode-specific scoring responded to reaching a target A2 15% greater than baseline walking. Learning outcomes were compared across groups with significance denoted as $p<0.05$. Results. Independent of group, participants increased A2 in response to real-time kinetic biofeedback ($p<0.001$). This increase was notable during learning (55% increase), throughout the washout (18%), and after a 24-hour break (19%) ($p's<0.001$). Learning performance differed between groups. Participants who received Positive feedback continued to increase A2 across the learning sessions, the other groups plateaued ($p=0.03$). Positive feedback also led to greater retention between sessions ($p=0.01$). Discussion. These results support the feasibility of using real-time kinetics as biofeedback to induce locomotor learning. Additionally, our data support the premise that Reward-based feedback leads to the greatest learning outcomes, which is consistent with extant motor learning studies and extends these findings to power-based locomotor learning. Future studies should investigate the utility of A2-based biofeedback to induce learning in stroke survivors.



Disclosures: D.R. Young: None. **T.E. McGuirk:** None. **E.S. Perry:** None. **W.M. Joiner:** None. **C. Patten:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.15

Topic: E.06. Posture and Gait

Support: JST-Mirai Program
JST CREST under Grant JPMJCR14E4

Title: The intrapersonal correlation between precompetitive physiological states and superior performance in extreme sports athletes

Authors: *S. MATSUMURA[1], K. WATANABE[2], S. MINAMI[1], N. SAIJO[1], Y. OOISHI[1], M. KASHINO[1];
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Abstract: Elite athletes in extreme sports, such as freestyle snowboarders, show sophisticated control of whole-body movement under immense competitive pressure. While cognitive pressure's effects on psychophysiological states and posture control are recognized, a previous study showed a positive relationship between precompetitive sympathetic predominance and subsequent jump performance in elite freestyle snowboarders. This finding suggests that elite snowboarders can transfer cognitive pressure into jump performance. However, whether all athletes have this skill remains unclear. This study investigated the intrapersonal relationship between precompetitive physiological states and performance. We measured the precompetitive physiological states of 20 elite freestyle snowboarders immediately before a jump attempt and their subsequent performance in the Snowboard Big Air competition. Three-minute measurements of electrocardiograms were taken ten minutes before jumps in *practice*, *competition*, and *post-competition* situations. We calculated mean heart rate (HR), low-frequency to high-frequency component ratio (LF/HF ratio) and the logarithm of the HF component of heart rate variability (HRV) as indices of physiological states. Three professional judges scored performances of all jumps based on international rules. The highest score in the *competition* session defined the rankings of the competition. The results indicated that the mean HR and LF/HF ratios of the HRV of athletes with high maximum scores had stronger positive correlations with the competition scores, while those with low maximum scores showed no or negative correlations. Furthermore, mean HR and LF/HF ratios of the HRV of athletes with high maximum scores more strongly positively correlated with amplitude score, an index used to evaluate jump height. This finding suggests that snowboarders who acquire high scores convert the effects of sympathetic predominance into better performance. Hence, we conclude that elite athletes may effectively convert the increase in sympathetic activity induced by high pressure into superior performance.

Disclosures: **S. Matsumura:** A. Employment/Salary (full or part-time):; NTT Communication Science Laboratories. **K. Watanabe:** None. **S. Minami:** A. Employment/Salary (full or part-time):; NTT Communication Science Laboratories. **N. Saijo:** A. Employment/Salary (full or part-time):; NTT Communication Science Laboratories. **Y. Ooishi:** A. Employment/Salary (full

or part-time):; NTT Communication Science Laboratories. **M. Kashino:** A. Employment/Salary (full or part-time):; NTT Communication Science Laboratories.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.16

Topic: E.06. Posture and Gait

Support: UK MRC (MR/T046619/1)
US ARO W911NF-15-1- 0358
Royal Society (UF120507)

Title: Biomechanics of limb motion: a function of size and frequency

Authors: *G. P. SUTTON[1], N. S. SZCZECINSKI[2], H. J. CHIEL[3], R. D. QUINN[4];
[1]Univ. of Lincoln, LINCOLN, United Kingdom; [2]West Virginia Univ., Morgantown, WV; [3]Biol., Case Western Res. Univ., University Heights, OH; [4]Mechanical Engin., Case Western Reserve Univ., Akron, OH

Abstract: During behavior, the work done by actuators on the body can increase the body's kinetic energy, increase the body's potential energy, or be dissipated as heat. Allocation of actuator work between these three forms of energy predicts the force output required to actuate a particular motion. The features and actuation of locomotion have been successfully predicted by nondimensional parameters that express the ratio between exactly two forms of energy. However, two animals of different sizes or two motions at different speeds may not share the same dominant forms of energy, complicating analysis. Thus, for broad comparison of locomotion across many orders of magnitude of limb length and cycle period, a dimensionless parameter that includes gravitational, inertial, elastic, and viscous forces is needed. This study proposes a nondimensional parameter that relates these four forces: the phase angle (phi) between the displacement of the limb and the actuator force that moves it. Using allometric scaling laws, for terrestrial walking is expressed as a function of the limb length and the cycle period at which the limb steps. This single parameter, phi, determines how energy is distributed within the step, thus determining the mechanical behaviour of the limb: viscous, inertial, or quasi-static. It also then determines the governing dimensionless parameters that govern that limb's mechanics.

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Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.17

Topic: E.06. Posture and Gait

Title: Application of Neuromuscular Electrical Stimulation during repeated perturbations can enhance motor adaptation among highly impaired people with chronic stroke.

Authors: *R. PUROHIT[1], S. WANG[2], G. VARAS-DIAZ[3], P. PATEL[4], J. FUNG[5], T. BHATT[2]; [2]Physical Therapy, [1]Univ. of Illinois at Chicago, Chicago, IL; [3]Univ. Finis Terrae, Santiago, Chile; [4]Colorado state university, Fort collins, CO; [5]Physical Therapy, Mcgill university, Montreal, QC, Canada

Abstract: Background: Previous studies have indicated that people with chronic stroke (PwCS) can acquire fall-resisting skills even after a single session of perturbation-based training (PBT). However, PwCS with high motor impairment could not adapt to higher perturbation intensities, thereby limiting the scalability of PBT. Thus, studies have suggested that highly impaired PwCS may need higher dosage or supplemental agents to adapt to high-intensity perturbations. Neuromuscular electrical stimulation (NMES), typically applied to the paretic muscle(s), is a supplemental agent clinically used to enhance functional mobility and gait in PwCS. Our recent study showed that NMES to paretic quadriceps during the onset of a high-intensity (acceleration: 12 m/s^2) stance-slip resulted in reduced laboratory falls and improved reactive balance responses compared to those without NMES. However, it is still unknown whether combined NMES and PBT compared to PBT alone could induce superior motor learning effects. Thus, this study examined the effect of a single session of PBT with and without NMES on reactive balance and fall-risk in highly-impaired PwCS. **Methods:** 16 PwCS with high-motor impairment (Chedoke McMaster Leg score: ≤3/7) were assigned to either PBT group (n=8) or NMES-PBT group (n=8). All participants received a pre and post-training stance-slip at high-intensity (acceleration: 12 m/s^2). The intervention for both groups included 11 consecutive stance-slips at a lower-intensity (acceleration: 7.5 m/s^2). In addition, the NMES-PBT group received electrical stimulation to paretic quadriceps within 50-500 millisecond after perturbation onset. Slip outcome (fall/no fall), proactive (pre-slip stability) and reactive balance (post-slip stability, compensatory step length, # of compensatory steps) were examined pre and post-training. **Results:** A 2x2 Analysis of variance showed main effect of group, trial and group x trial interaction ($p<0.05$) on slip outcome, post-slip stability, compensatory step length and # of compensatory steps. Post-hoc comparisons showed no group differences in all variables during pre-training ($p>0.05$). Post-training, NMES-PBT showed greater reduction in falls, increase in post-slip stability, increase in compensatory step length and reduction in # of compensatory steps over PBT alone ($p<0.05$). **Conclusion:** Application of NMES to paretic knee extensors during single-session of PBT has superior benefits on reactive balance and fall-risk but not on proactive balance control over PBT alone. Future studies may need to examine the short and long-term neuro-modulatory effect of NMES on reactive balance responses in this population.

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Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.18

Topic: E.06. Posture and Gait

Support: NIH T32NS082128-06
NIH R21NS119849

Title: Performance fatigability in essential tremor

Authors: *B. YACOUBI KEYHANI[1], S. DELMAS[1], Y. CHOI[1], J. KIM[1], J. HUBBARD[1], M. S. OKUN[2], E. A. CHRISTOU[1];
[1]Applied Physiol. and Kinesiology, [2]Neurol., Univ. of Florida, 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, and approximately 30%-50% experience subjective fatigue, which is defined as an overwhelming sense of tiredness, lack of energy or need for increased effort. However, little is known on objective fatigability in ET, which is thought to contribute to gait and balance deficits in other neurologic conditions. Performance fatigability is defined as an objective decline in performance measurable during motor or cognitive tasks. Here we conducted a pilot study to examine the effect of neuromodulation on performance fatigability of 7 ET patients undergoing VIM DBS therapy. We compared performance fatigability between DBS OFF and ON conditions during an arm raise and leg raise seated postural task, and during an abdominal contraction postural task. We quantified the onset of fatigue for the deltoid, rectus femoris, and rectus abdominis muscles, as the inflection point at which a steady increase in smoothed EMG activity occurred. Fatigue onset was significantly ($p<0.01$) delayed in the DBS ON condition relative to the DBS OFF condition across all three muscles. Interestingly, greater VIM DBS-induced reduction of performance fatigability associated with suppression of muscle activity from 35-60 Hz but not 4-8 Hz, the tremor frequency band in ET. This suggests that neuromodulation of fatigability with VIM DBS may be distinct from tremor suppression. Finally, we found that VIM DBS-induced reduction of performance fatigability associated with improvements in mobility and balance measures, suggesting that neuromodulation of performance fatigability may be a means to address these deficits in ET.

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Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.19

Topic: E.06. Posture and Gait

Support: Bell Family Endowed Chair
TL1 TR000441

Title: Walking and turning on an omnidirectional treadmill in virtual reality differs from overground gait in healthy young adults

Authors: *M. MCGRATH[1,3], C. WALTZ[1], A. ROSENFELDT[1], K. OWEN[1], K. HASTILOW[1], L. SCELINA[1], K. SCELINA[1], M. KOOP[1], J. ALBERTS[1,2];
[1]Biomed. Engin., [2]Neurolog. Institute, Ctr. for Neurolog. Restoration, Cleveland Clin., Cleveland, OH; [3]Sch. of Med., Case Western Reserve Univ., Cleveland, OH

Abstract: Background: Virtual reality (VR) is a powerful yet underutilized tool with countless applications in clinical medicine, including the diagnosis and treatment of neurological disease. Until recently, virtual reality applications were limited by the locomotion problem – moving through a virtual world while the body remains stationary can trigger symptoms of motion sickness. Omnidirectional treadmills (ODTs) allow real walking for virtual navigation, providing a fully immersive VR experience and reducing the risk of VR-related sickness. Coupling ODTs with VR headsets can facilitate the full utilization of virtual reality applications in medicine, including quantification of walking and turning in complex environments. Before VR locomotion metrics can be meaningfully interpreted in neurologic populations, it is necessary to characterize the relationship between overground walking and VR ODT walking.
Purpose: The objective of this study was to establish how the spatiotemporal and kinematic signatures of gait differ during walking and turning tasks completed 1) overground and 2) on an ODT in a VR environment.
Methods: A sample of 15 healthy young adults between the ages of 18 and 30 years participated in the study (8 male, 7 female; average age 25.1 ± 4.0 years). All participants completed forward walking, 180-turn, and 360-turn tasks overground and on an ODT while wearing an immersive VR headset. The VR environment replicated the physical testing space. Full-body motion capture was used to quantify position and orientation throughout each trial.
Results: In the ODT + VR condition, participants walked at a slower speed compared to overground (0.34 m/s vs 1.3 m/s), with a slower cadence, shorter step lengths, and longer step times. These differences were reflected in kinematic joint metrics, including reduced range of motion of the hip, knee, and ankle. Compared to overground, both 180-degree and 360-degree turns on the ODT took longer (180 turns: 6.3 ± 0.7 s vs. 2.2 ± 0.2 s, 360 turns: 10.1 ± 1.5 s vs. 3.7 ± 0.5 s) and required more steps to complete (180 turns: 10.6 ± 1.6 vs. 4.9 ± 0.5; 360 turns: 17.4 ± 2.9 vs. 7.3 0.8).
Conclusions: Omnidirectional treadmill walking offers a potential solution to the locomotion problem in VR, allowing users to naturally navigate a full-scale virtual environment. However, biomechanical outcomes from ODTs must be interpreted carefully, as ODT walking differs from overground walking across spatiotemporal and kinematic parameters in healthy young adults.

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Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.20

Topic: E.06. Posture and Gait

Title: Type of cognitive task matters during dual-task reactive balance

Authors: *J. PITTS[1], L. KANNAN[1], T. SZTURM[2], T. S. BHATT[1];
[1]Physical Therapy, Univ. of Illinois at Chicago, Chicago, IL; [2]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Balance is an attentionally-demanding task. Most previous dual-task (DT) studies have examined the effect of a concurrent cognitive task on standing balance or gait rather than the ability to recover from perturbations (i.e., reactive balance). Further, DT studies have mainly included only one cognitive task, leaving it unknown if tasks involving different domains have different effects on reactive balance. This study examined how four cognitive tasks affected reactive balance responses and cognitive performance during DT slipping. Fifteen young adults (25.8(4.3) y) were exposed to unexpected standing treadmill-slips alone (motor single-task (ST)) and while completing four different cognitive tasks in random order: 1) *Target Game*: Participants controlled a computer mouse by turning their head to catch a vertically-dropped ball on a screen; 2) *Tracking Game*: Participants moved their head to track a horizontally-moving target; 3) *Auditory Clock Test (ACT)*: Participants heard a time and identified if the hour and minute hands were on the same or opposite side of the clock face; 4) *Letter Number Sequencing (LNS):* Participants heard a letter and number pair (e.g., A1) and continued the sequence (e.g., B2, C3). Each task lasted for 45 seconds, during which three slips were delivered. Participants also completed cognitive tasks without slipping (cognitive ST). Reactive balance outcomes (margin of stability at recovery step touchdown, recovery step length, reaction time) were compared between motor ST and DT conditions using one-way repeated measures ANOVA. Cognitive performance on each task was compared between cognitive ST and DT using paired t-tests. There were no significant differences in reactive balance outcomes between ST and DT slips ($p>0.05$), indicating that the ability to recover balance was not affected by any cognitive task. Therefore, young adults may prioritize their motor response during DT slipping to prevent falling. Further, there was a significant decrease in cognitive performance on the Target (hit rate: 98% vs. 88%, $p=0.002$) and Tracking (sum of errors: 265 vs. 397, $p<0.001$) games during DT, but no difference on the ACT or LNS ($p>0.05$). Thus, there could be a common overlap between the cognitive resources used for reactive balance and visuomotor processing, such that limited resources are available for optimal performance on both tasks when completed simultaneously. Overall, our results show that tasks involving different cognitive domains can have different

effects on performance during DT reactive balance. Future DT studies will help further the understanding of which cognitive domains play a role in reactive balance control.

Disclosures: **J. Pitts:** None. **L. Kannan:** None. **T. Szturm:** None. **T.S. Bhatt:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.21

Topic: E.06. Posture and Gait

Support: NIH Grant 90084097

Title: Patterns of neck muscle activation in rhesus monkeys during walking

Authors: *R. WEI, O. R. STANLEY, K. E. CULLEN;
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Abstract: Sensory inputs arising from visual, vestibular, and muscular receptors play a crucial role in successful locomotion. Vestibulo-spinal reflexes make an essential contribution to locomotion by compensating for head-on-body movement to stabilize head and body posture relative to space. Notably, the vestibulocollic reflex (VCR) activates neck muscles to maintain the orientation of the head relative to the torso. Previous studies have shown that the head is well-stabilized in both pitch and roll by the VCR during quadrupedal locomotion in monkeys. However, no work to date has directly examined neck muscle activity during walking. Accordingly, using a combination of head and body 3D motion capture and acute intramuscular EMG recording, we studied head stabilization and the functional activity of neck muscles during walking in two rhesus monkeys with intact vestibular systems and compared these results with those from a bilateral vestibular loss monkey. A head-mounted 6D gyroscope/accelerometer was used to record head motion, while 6 high-speed cameras were used for synchronized motion recording. We use DeepLabCut - an open-source markerless pose estimation system - to extract the animals' 3D posture for gait analysis. Separately, an open-source, marker-based tracking system was used to extract the animals' head and trunk 6D positions. Acute intramuscular EMG recordings were performed bilaterally in the splenius capitis (SPL) muscles, from which single motor unit activity was identified. We found that the head was well-stabilized in healthy monkeys, with head-on-body movement compensating for the movements of the body. Both left and right SPL EMG and single motor unit activity showed antagonistic phase-dependent activity during locomotion. In contrast, without vestibular sensory input, the bilateral vestibular loss monkey showed much larger head oscillations, which did not compensate for the swings of the body. In this animal, neck muscles did not show phase-locked activity or antagonism. Taken together, our results provide new insight into the unique phasic pattern of muscle activity generated by the VCR to enhance head stability during locomotion, and establish that vestibular

sensory inputs play a key role in organizing this activity to ensure compensatory head movement during locomotion.

Disclosures: R. Wei: None. **O.R. Stanley:** None. **K.E. Cullen:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.22

Topic: E.06. Posture and Gait

Support: R01-DC002390
R01-DC018061
1UF1NS111695-01

Title: Quantifying the effects of vestibular schwannoma on head-on-trunk movement kinematics.

Authors: *R. ARYAN[1], O. ZOBEIRI[4], L. WANG[1], J. MILLAR[2], M. SCHUBERT[2], K. CULLEN[3];
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Abstract: Individuals with vestibular schwannoma (VS) experience impaired balance and gait control. However, to date, surprisingly little is known about the impact of vestibular loss on head-on-trunk stability in VS, particularly during challenging balance and gait activities. Assessment of head-on-trunk kinematics before and after surgery provides clinical practice with important information to facilitate compensation and recovery of posture and gait in VS. Accordingly, the goal of the present study was to a) identify differences between head-on-trunk kinematics in people with VS and healthy controls; b) explore changes in movement kinematics following tumor resection; c) examine association among the head-on-trunk kinematics and clinical measures.**Methods:** Nine participants with VS and 9 healthy controls (mean ages= 56 and 49 years, respectively) were asked to keep their balance while doing multiple challenging balance and gait tasks. Patients were tested before (pre-op) and 6 weeks post-surgery (post-op). Range and standard deviation (SD) of head-on-trunk linear accelerations (mediolateral, anteroposterior, vertical) and angular velocities (yaw, pitch, roll) were measured via 2 inertial measurement units that were placed on the head, and on the trunk approximately at the level of body's centre of mass. Balance and mobility functions were examined via performance-based scales and questionnaires; integrity of vestibular functions was tested via the digital visual acuity and video head impulse tests.**Results:** Compared to controls, both pre and post-op groups demonstrated more variability (greater SD) of head-on-trunk kinematics in multiple tasks, in particular in tasks with narrow or unstable base of support and with eyes closed (p value<0.05). However, no significant differences in head-on-trunk kinematics were observed between pre and

post-op groups (p value>0.05). Additionally, for both pre and post-op groups, head kinematics were more consistently correlated with the trunk kinematics, mostly in tasks with unstable base of support and eyes closed. Finally, correlational analysis of clinical measures with head-on-trunk kinematics revealed significant correlations in patients groups in tasks with unstable base of support and eyes closed.**Conclusions:** Overall our finding demonstrate that head-on-trunk control is impaired in VS, particularly in situations with decreased sensory information. Greater correlations between the head and trunk movements are consistent with the decreased efficacy of descending vestibular neck control mechanisms. Such impairments, in turn, result in the reduction of stabilizing head-on-trunk movements in VS.

Disclosures: R. Aryan: None. **O. Zobeiri:** None. **L. Wang:** None. **J. Millar:** None. **M. Schubert:** None. **K. Cullen:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.23

Topic: E.06. Posture and Gait

Title: Use of actual and remembered visual feedback for obstacle clearance during walking

Authors: T. WATTS[1], M. CANNON[2], A. G. ADEYEMO[1], T. GAUSS[1], *J. M. HONDZINSKI[1];
[1]Kinesiology, Louisiana State Univ., Baton Rouge, LA; [2]St. Joseph's Acad., Baton Rouge, LA

Abstract: We previously showed that the application of illusory vertical lines on the raised surface of the first of two obstacles differed from its application to the second of two obstacles such that people used a greater toe clearance over both obstacles when presented with the illusory obstacle first. Participants also used visual feedback from the first obstacle for toe clearance over the second obstacle. We expanded upon these results by exploring whether people would perform similarly if asked to step over a remembered first obstacle. Specifically, we determined if removal of the first obstacle with an illusory surface rise still produced greater toe clearance when people stepped over its remembered location and when they stepped over a second obstacle. Participants stepped over two obstacles (34.2 cm x 12 cm x 23.6 cm shoe boxes), one with a plain and one with an illusory (vertical lines) surface on the rise of the box while walking across a room at a comfortable walking speed. Boxes were spaced according to the average step length of each participant while walking at a comfortable speed so that they used the dominant leg for obstacle clearance with one non-dominant leg step between the two boxes. For remembered trials, the first box was removed at the time of the last dominant leg step prior to the obstacle location. Movements of the body were monitored using a four-camera passive marker Qualisys system (Qualisys Medical AB, SE) and toe height above the floor was calculated and used as the primary variable of interest. Average toe height of four trials for each condition were compared using repeated measures ANOVAs with box order (first, second), box

type (plain, illusory), and clearance type (real, remembered) as within subject factors ($p < 0.05$). Results revealed outcomes similar to those observed previously for real trials, in which participants used greater toe height stepping over both obstacles when presented with the illusory obstacle first. Interestingly, toe height for real trials also exceeded toe height for remembered trials (as much as two-fold in some cases). In some trials participants did not always step over the remembered box location and would have hit it if it remained in place. Moreover, participants did not always achieve a greater toe height when stepping over the plain obstacle subsequent to stepping over the remembered illusory obstacle compared to stepping over the illusory obstacle subsequent to stepping over the remembered plain obstacle. These data provide evidence that people can use visual feedback of object properties just prior to obstacle avoidance during typical walking gait.

Disclosures: T. Watts: None. **M. Cannon:** None. **A.G. Adeyemo:** None. **T. Gauss:** None. **J.M. Hondzinski:** None.

Poster

643. Posture and Gait: Kinematics, Biomechanics, Exercise, and Fatigue I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 643.24

Topic: E.06. Posture and Gait

Title: Role of arm swing in gait adaptability in young and older adults

Authors: *S. SUN, T. AMMIRATI, M. SMITH, T. STAKER;
Physical Therapy, Alvernia Univ., Reading, PA

Abstract: Rhythmic arm swing occurs during gait in humans. While it has been proposed that the arm swing occurs through active and passive mechanisms, it is known to decrease metabolic cost and energy expenditure, and increase balance and stability during gait. Nonetheless, it is yet to be understood whether the arm swing plays a role in gait adaptation that involves modification of gait patterns to meet the environmental demands. Further, while gait adaptability is known to decline in older adults, it is unclear whether the arm swing, also known to be altered with aging, contributes to the differences in gait adaptability in older adults. To this end, the aim of this study was to examine the role of arm swing in gait adaptation in healthy young and older adults using split-belt treadmill paradigm. We hypothesized that altered arm swing delays adaptation to the asymmetric walking pattern, and that the restricted arm swing differently affects gait adaptation between young and older adults. We recruited 12 healthy individuals (7 young adults, mean age 24.17 yrs; 5 older adults, mean age 66.45 yrs), and collected kinematic data using reflective markers placed on the upper and lower extremities (Qualisys, Sweden). After a short familiarization session on the treadmill (Bertec, Columbus, OH), participants performed a classic split-belt paradigm: 5 minutes with belts tied (0.5 m/s; *baseline* condition), followed by 10 minutes with split-belts (0.5 and 1.5 m/s; *adaptation* condition), followed by 5 minutes of walking with belts tied (0.5 m/s; *post-adaptation* condition). The protocol was performed twice

with different arm swing conditions: normal/unrestricted and restricted arm swing. The order of the arm swing conditions was randomized for each participant. The study protocol was approved by Alvernia University Institutional Review Board. Our preliminary analysis of the time it took to achieve step length symmetry between the unrestricted and restricted arm swing conditions indicated that older adults were slower to achieve step length symmetry during the adaptation and post-adaptation conditions, consistent with the previous findings. Interestingly, the restricted arm swing did not alter the time to achieve step length symmetry, similarly in both young and older adults. This may be attributable to the slow walking speed on the treadmill, which may not have provided a significant challenge to the locomotor system to utilize the arm swing and enhance the gait adaptation. The future study will determine if faster walking speed on the treadmill results in differences in gait adaptation patterns depending on the arm swing conditions in young and older adults.

Disclosures: S. Sun: None. **T. Ammirati:** None. **M. Smith:** None. **T. Staker:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.01

Topic: F.01. Neuroethology

Support: NIH-NINDS Grant R01NS054898

Title: Active neural coordination of motor behaviors with internal states

Authors: *Y. ZHANG[1], D. Y. TAKAHASHI[2,1], A. EL HADY[3,4,1], D. A. LIAO[1], A. A. GHAZANFAR[1];
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Abstract: Homeostasis is maintained through the interplay between internal physiological states and sensorimotor interactions with the external environment. What is the role of the brain in coordinating these internal and external activities? We investigated this question in the condition where an animal was behaving without any salient sensory cues. One possibility is that, in this context, brain activity is simply driven by interoceptive and proprioceptive signals. Alternatively, the brain is actively coordinating internal and external activities. To test these hypotheses, we applied functional ultrasound imaging in a large medial sagittal section of the brain--multiple cortical and subcortical areas--in marmoset monkeys while monitoring their spontaneous movements and cardiac activity. We then evaluated the directed information flow between each pair of signals among brain activity, heart rate, and movement based on causal inference in the frequency domain. We found that information flowing from the brain to heart rate fluctuations

and movements was significantly greater than in the opposite direction, suggesting that the brain is actively coordinating internal states with external movements. By analyzing the functional connectivity using information flow between brain regions, we also found that the areas involved in internal versus external activity were spatially distinct but extensively interconnected. Linking the regional activity to global brain state transitions, we identified an alternating pattern between internal and external regulation.

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Poster

644. Neuroethology: Sensory Motor Systems I

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Program #/Poster #: 644.02

Topic: F.01. Neuroethology

Support: NIH grant 5T32AA007456-39
NIH grant 5R01AA022977-08

Title: Females exhibit heavier binge alcohol use than male rats in a model of voluntary escalation and maintenance of alcohol dependence

Authors: *A. AVELAR[1], K. NARULA[2], K. QUON-ADAMS[2], S. KRAUSE[3], A. J. ROBERTS[4], G. DE GUGLIELMO[5], O. GEORGE[6];
[1]Univ. of California San Diego, San Diego, CA; [2]Univ. of California San Diego, La Jolla, CA; [4]Animal Models Core, [3]The Scripps Res. Inst., La Jolla, CA; [5]Psychiatry, Scripps Res. Inst., San Diego, CA; [6]department of psychiatry, UCSD, La Jolla, CA

Abstract: Alcohol misuse has long been a serious health and social concern. Alcohol binge use can increase escalation of alcohol use in humans, and National Institute on Alcohol Abuse and Alcoholism (NIAAA) has described high intensity drinking which is consuming twice the alcohol as a binge user in a 2 hour period. Binge and high intensity drinking increase negative health and social outcomes for humans, however there are few animal models exhibiting voluntary self-administration of similar amounts of alcohol. Previous work shows that male rats escalate self-administration of ethanol vapor to the point of becoming dependent and that ~25% of male Wistar rats binge ethanol vapor to reach blood alcohol levels (BALs) in the 300-450 mg% range (de Guglielmo et al., 2017, George lab grant). However, this study was performed only in male rats and it is unclear if the same phenomenon occurs in females. To address this gap, we evaluated sex differences in ethanol vapor self-administration (EVSA) in Wistar rats. Rats were tested under a fixed ratio 1 schedule of reinforcement for 8 hr sessions every other day and the duration of alcohol vapor puff increased every 8 sessions from 2, to 5, to 10 minutes. Tail blood was collected throughout the experiment and BALs were measured by gas chromatography. Female rats learned to discriminate between active and inactive nosepokes

earlier and more accurately than males. Female rats self-administered more ethanol vapor than males. BALs increased throughout the ethanol vapor sessions, with female levels being higher than males. About 21% of ethanol binge rats were female and ~7% male, as defined by rats having a BAL higher than average + 2SD. Overall, the present results show that female rats discriminate better between active and inactive nosepokes, have faster acquisition of ethanol vapor self-administration, and binge more than males. These results demonstrate the relevance of the ethanol vapor self-administration model to study binge ethanol use and demonstrate that females are particularly vulnerable to binge use of alcohol.
Referencesde Guglielmo G, Kallupi M, Cole MD, George O. Voluntary induction and maintenance of alcohol dependence in rats using alcohol vapor self-administration. Psychopharmacology (Berl). 2017 Jul;234(13):2009-2018. doi: 10.1007/s00213-017-4608-7. Epub 2017 Mar 24. PMID: 28342089; PMCID: PMC5658208.
George lab grant unpublished data
https://arcr.niaaa.nih.gov/binge-drinking-predictors-patterns-and-consequences/high-intensity-drinking

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Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.03

Topic: F.01. Neuroethology

Support: NIH Grant R01 NS118
NSF Grand DB12015317, IOS1754869

Title: When speed is nearly independent of size: quasi-static mechanics of cyclic Aplysia feeding

Authors: *A. SKALSKI DE CAMPOS[1], I. KAZA[1], K. WANG[2], V. FAN[2], J. P. GILL[1], H. J. CHIEL[1], G. P. SUTTON[3];
[1]Biol., [2]Case Western Reserve Univ., Cleveland, OH; [3]Lincoln Univ., Lincoln, United Kingdom

Abstract: How does the size of an animal performing a rhythmic behavior affect the speed and magnitude of the behavior? To address this question, we studied biting and swallowing in the marine mollusk *Aplysia californica* over a range of 8 to 1424 grams. We hypothesized that the feeding apparatus, the buccal mass, might grow isometrically with animal mass. Furthermore, because the behavior is slow and the animal mass is small, we hypothesized that the behavior would be quasi-static, i.e., dominated by elastic forces. In turn, this suggested that the duration of the behavior (biting or swallowing) would be constant over a range of animal sizes.
To test these hypotheses, duration of loaded and unloaded swallows, peak swallowing force, and

mass of the animal's feeding apparatus were measured. Animals were deprived of food to increase appetite for a duration dependent on their mass. Small animals (under 100 g) were starved for 3-5 days, medium to large animals (100 g+) were starved for 7-9 days. An active cooling system maintained water temperature at 16.0 0.1°C. Bits of the seaweed nori placed in "chopsticks", regular strips of nori, two-sided tape covered with nori and a force transducer were used to acquire data on bite duration, duration of unloaded and loaded swallows, and force generated by the animals, respectively. Behavior was videoed, and the feeding apparatus mass was measured after recording behavior.
We have discovered that there was a change in the scaling of the growth of the buccal mass relative to animal mass after the animals were larger than 160 grams, corresponding to sexual maturity (CITE). When juveniles were plotted separately from animals greater than 160 grams, both groups showed isometric scaling of the buccal mass. The duration of inward movements during swallowing were essentially independent of animal mass, as predicted. In contrast, the duration of juvenile biting protractions increased with size, but after sexual maturity the durations were again independent of animal size.
These data suggest that changes in the musculature's anatomy or physiology during the juvenile phase may change the duration of the behavior, but for much of its lifespan, feeding behavior is primarily dominated by elastic forces, which may have interesting implications for its neural control.

Disclosures: A. Skalski De Campos: None. **I. Kaza:** None. **K. Wang:** None. **V. Fan:** None. **J.P. Gill:** None. **H.J. Chiel:** None. **G.P. Sutton:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.04

Topic: F.01. Neuroethology

Support: NASA Cooperative Agreement NNX16AO69A

Title: The Great Divide: Lab to Field Neural Recordings

Authors: *M. GAIDICA, B. DANTZER;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Animal-borne sensors have a long history of revealing interesting behaviors and adaptations to the rigors of the natural world. For example, sleep can occur mid-flight in birds, one hemisphere at a time in dolphins, and while ruminants are standing upright. However, progress towards probing complex, neurophysiological behaviors like sleep in free-ranging animals has been slow-paced due to the burden of surgical intervention and device limitations. To address the growing need for a multipurpose "biologging" device that can be implanted and deployed for many weeks, we developed a coin-sized, low-cost, wireless neurophysiology

platform and demonstrated its use in rats and squirrels to detect sleep states and waking behavioral motifs. We provide special considerations for our biologger's use across many species and environments and explore its closed-loop capabilities by performing high-speed, on-board filtering of neural data while cueing external hardware using a low-latency wireless link. Overall, progress in this direction is needed to bridge the 'great divide' between what we know about behavior and physiology in the lab to more natural environments and non-traditional animals.

Disclosures: M. Gaidica: None. **B. Dantzer:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.05

Topic: F.01. Neuroethology

Support: Wellesley College
National Science Foundation IOS-2016188

Title: Acute water deprivation differentially alters the perception of hygrosensory and visual cues that support in-flight hygrotaxis behavior across Drosophila species

Authors: D. L. LIMBANIA[1], C. ZHU[2], G. TURNER[2], S. M. WASSERMAN[2];
[1]UCLA, Los Angeles, CA; [2]Neurosci. Dept., Wellesley Col., Wellesley, MA

Abstract: Organisms must integrate internal and external states to generate behaviors that support survival. Our work investigates how dynamic representations of sensory information underlie flexible behavioral outputs in varied internal states. For example, due to their large surface area to volume ratio, dehydration is a persistent threat to the cosmopolitan fruit fly, Drosophila melanogaster (D. mel.). We utilize a virtual reality flight simulator that permits flying flies to rotate freely in response to controlled sensory stimuli to ask whether D. mel. alter the value of sensory cues that signal the presence of water when in a dehydrated state. We find that while well-hydrated flies assign a neutral value to and thus ignore a humid air plume in flight, acutely dehydrated (3 hrs) flies assign a positive value to the same humid air plume in order to generate water-seeking behavior. In addition to modifying in-flight hygrotaxis behavior, we also find hydration-state dependent changes in response to visual indicators of water. Finally, we show that state-dependent changes in hygrotaxis behavior vary across Drosophila species. These findings provide a foundation for further examination of (1) how neural circuits in the brain integrate internal physiological state to modify perception across sensory modalities to reliably generate contextually appropriate behavior and (2) how these circuits might have evolved differently to support Drosophila in varied external environments.

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Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

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Program #/Poster #: 644.06

Topic: F.01. Neuroethology

Support: MURI N00014-19-1-2373

Title: Modeling Octopus Arm-Sucker Sensory-Motor Coordination

Authors: *J. CUI[1,2], E. GRIBKOVA[1,2], R. GILLETTE[1,3];
[1]Neurosci. Program, [2]Coordinated Sci. Lab., [3]Dept. of Mol. and Integrative Physiol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Octopus' arms and suckers are notable for flexibility and actions semi-independently of the central brain. While neuronal mechanisms for arm-sucker coordination are yet poorly understood, we are modeling testable sensory-motor networks based on behavioral observations. A present model simulates the sensory-motor network of the arm by connecting several segments (joints) in tandem. Each joint mimics the brachial ganglia and suckers of the arms and incorporates a three-layered neural network. The outermost layer consists of chemosensory neurons whose excitation is a function of stimulus intensity. Postsynaptic interneurons sharpen the signal in a lateral inhibitory network and send it to core motor neurons. The ganglia predict the direction of the stimulus source and orient towards or away, based on the firing rate of the core neurons from each direction and the motivational state. Each joint is synaptically connected to neighboring joints to form a network along the arm. Lateral inhibition and hierarchical control among joints and arms coordinate the movement. This preliminary model can direct the arm to search for an odor stimulus source up the concentration gradient, an ability useful in real-world foraging scenarios where the target's location is hidden from sight. The model's second function is generation of sensory maps. Multiplying the direction of each core neuron in the sensory network by its firing rate and summing the neural activity from each direction allows the sensory system to estimate both direction and distance of the stimulus source. This simple working model is both testable and modifiable based on experimental data. It shows how complex behaviors of octopuses' arms can be derived from simple peripheral neural interactions without moment-to-moment direct input from the CNS.

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Poster

644. Neuroethology: Sensory Motor Systems I

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.07

Topic: F.01. Neuroethology

Support: Christopher Newport Univ. 2021 Summer Scholars Program

Title: Testing social preference in zebrafish (Danio rerio) across both discrete-choice and free-operant procedures using the instrumental-response T-maze and the open-tank free-swim task

Authors: *A. J. VELKEY, II[1], S. NEKKANTI[1], A. SAPRE[2], K. LANDRY[3], K. TOMLIN[1]; [1]Neurosci., [2]Mol. Biol. and Chem., [3]Organismal and Envrn. Biol., Christopher Newport Univ., Newport News, VA

Abstract: Adult zebrafish (*Danio rerio*) display complex social behaviors and are used as an animal model for neuropsychiatric diseases and disorders, including research on zebrafish behavioral responses to social cues. Previous research indicates that zebrafish prefer social interaction with live fish stimuli over stimuli of lower fidelity (e.g. video or animatronic models), and a variety studies have been conducted on social preference in zebrafish using several techniques including the operant open-tank free swim task as well as the instrumental discrete-choice response task. However, no researchers have yet reported whether zebrafish demonstrate a preference between a mirrored self-reflection and a live fish stimulus in a discrete-choice instrumental response task, and no reported research has compared preference across both tasks

to determine if the social preference remains consistent. In the current study, adult zebrafish were first tested for their social preference between a mirrored self- reflection and a live conspecific as alternative rewards for swimming to the terminus of a T-maze using the instrumental discrete-choice response task. While all subjects responded favorably to the alternative choices and demonstrated reduced response times across trials, roughly a third of subjects did not demonstrate a clear preference for one reward over the other, while nearly a third of the other subjects demonstrated a preference for the mirror reward and the remaining subjects demonstrated a preference for the live conspecific reward. Subjects were then tested using the operant open-tank free swim task with a different live conspecific presented on one end of the test chamber and a mirror presented at the other end. All of the trials for the open-swim free operant task were digitally recorded and movement paths for each subject were subsequently analyzed using EthoVision XT 10. These results were then used to compare the stability of preference across the two tasks, with mixed results. These findings can be used to further understand the mechanisms that elicit social responding in zebrafish and to determine how preference for a particular social reward remains stable across both the discrete-choice instrumental response task and the open-swim free operant task.

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Poster

644. Neuroethology: Sensory Motor Systems I

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Program #/Poster #: 644.08

Topic: F.01. Neuroethology

Support: NIH R01 EY031972
NIH R01 DC004154
NIH F32 DC018458

Title: Effect of post-training sensory input on systems consolidation of a motor skill

Authors: *T. STAY, E. CHAN, S. KIM, V. XIN, A. SOMERA, D. JANG, J. RAYMOND; Stanford Univ., Stanford, CA

Abstract: Systems consolidation is the process whereby the way a memory is stored is transformed over time. In oculomotor tasks, the expression of a learned eye movement response to a vestibular or visual input requires the cerebellar flocculus immediately after training, but not 24 hours later. A hypothesis is that plasticity in the flocculus during training alters the activity of the sole output neurons of the flocculus, which, in turn, triggers secondary, persistent plasticity in the downstream vestibular nucleus. Previous studies indicate that modifications in the vestibular nucleus occur within four hours after oculomotor learning. We tested how manipulation of the sensory input to the flocculus and vestibular nucleus during the post-training consolidation

period modify the amount of learning that was retained at 24 hrs. Modeling suggested that vestibular afferent activity might increase the amount of oculomotor memory consolidation relative to no vestibular activity. Accordingly, we trained three groups of mice to make larger eye movements in response to a vestibular input, i.e., to increase the gain of the vestibulo-ocular reflex (VOR), and then gave each group a different level of vestibular input during the post-training period. One group was returned to their home cages immediately after VOR training. A second group was head-restrained on a stationary vestibular turntable for one hour after training, to minimize vestibular input. The third group was head-restrained and given continuous 1-Hz sinusoidal passive vestibular stimulation for one hour after training. All three groups were tested for retention of the VOR-increase learning at 1 hour after training, then kept in darkness overnight and tested again for retention at 24-hours. We found that while the home cage group showed high levels of retention of VOR-increase learning at 24 hours, the other two groups showed significantly impaired retention of learning. These results identify a behavioral manipulation that can be used to analyze the patterns of neural activity in the VOR circuit that are necessary and sufficient to support the systems consolidation process.

Disclosures: T. Stay: None. **E. Chan:** None. **S. Kim:** None. **V. Xin:** None. **A. Somera:** None. **D. Jang:** None. **J. Raymond:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.09

Topic: F.01. Neuroethology

Title: A neuroethological approach to study zebrafish neurobiology using novel cone snail venom

Authors: *P. FLOREZ SALCEDO[1], B. M. OLIVERA[2], H. SAFAVI-HEMAMI[3,4], A. D. DOUGLASS[1];
[1]Neurobio., [2]Biol. Sci., [3]Biochem., Univ. of Utah, Salt Lake City, UT; [4]Biomed. Sci., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Cone snail venom has captivated the attention of the scientific community due to its potency, selectivity, and efficacy. Historically, conotoxin research has focused mainly on harnessing bioactivity to develop drug leads and pharmacological agents. However, little effort has been made to thoroughly understand the activities of these peptides in their native context, leaving important aspects of their biological function unexplored. We developed a new approach that combines the biochemistry of fish-hunting cone snail venom with the neurobiology of the zebrafish. We present the discovery of a new class of conotoxins from deep-water fish-hunting cone snails that target sensory neurons and disrupt the locomotion of the zebrafish. This work links the substantial knowledge of the chemistry of natural products with neuroethology to shed light on the molecular, cellular, and circuit aspects that control behavioral changes in the fish.

Roughly 150 species of fish hunting cone snails have been identified since the initial discovery of cone snail envenomation in 1956. Here we investigated the venom of two deep-water fish-hunting cone snails, *Conus rolani* and *Conus neocostatus,* using proteomics and transcriptomics and identified four novel conotoxins. These conotoxins were found to be highly expressed in the venom and do not share any sequence homology with previously known conotoxins. We further examined the bioactivity of these novel toxins using a series of behavioral paradigms performed on adult and larvae zebrafish. We found that these novel toxins significantly reduced the zebrafish locomotion as well as a diminished responsiveness to painful stimuli, highlighting the role of these toxins in predation. We utilized genetic labeling strategies and performed calcium imaging (*in-vivo*, and *in-vitro)* to dissect the bioactivity of these novel toxins in the zebrafish. Our results show that these new toxins silence the calcium activity of a subpopulation of sensory neurons by modulating the L-type calcium channels expressed in the sensory neurons. Together, these results uncover an important component of the venom arsenal of deep-water fish-hunting cone snails, while simultaneously revealing the functional roles of L-type calcium channels expressed in the sensory neurons for modulating locomotor behavior in the fish prey.

Disclosures: P. Florez Salcedo: None. **B.M. Olivera:** None. **H. Safavi-Hemami:** None. **A.D. Douglass:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.10

Topic: F.01. Neuroethology

Support: DFG Grant EXC 2117 – 42203798

Title: Hopper by name, hopper by nature. The state-dependent startle response of the desert locust.

Authors: *Y. GÜNZEL[1,2], H. KÜBLER[1], E. COUZIN-FUCHS[1,2];
[1]Neurobio., [2]Ctr. for the Advanced Study of Collective Behaviour, Univ. of Konstanz, Konstanz, Germany

Abstract: Swarms of the migratory desert locust can extend over several hundred square kilometres, and starvation compels this ancient pest to devour everything on its path. However, despite the plague's enormous socio-economic impact, estimated to affect ten per cent of humanity, only little is known about their collective decision-making processes. Here we intend to shed light on these by combining eye-opening field observations, controlled lab experiments, and fine-scale recordings from descending neurons. Deciding where to go and detecting danger along the way is crucial for survival. In the case of locust marching bands, animals in the swarm's periphery have higher access to environmental cues and, therefore, potentially higher means to steer the swarm towards food sources and away from threats. Our field data revealed

that peripheral animals were the first to startle in response to threatening stimuli. Moreover, their marching pattern included more stationary periods (standing bouts) than central animals. Since standing bouts have been associated with direction change, and hence potentially decision-making processes, we tested in the lab the apparent correlation between motion state (walking vs standing) and an animal's probability of startling. We show that standing locusts respond more reliably than walking ones, but animals of both motion states have similar response thresholds. After establishing this behavioural evidence, we now investigate whether the change in response reliability results from state-dependent modulation in descending motion detection neurons. Lastly, we transfer our findings on individual animals back to the group context by simulating swarms in a virtual reality environment and conducting large-scale group experiments. Altogether, our observations highlight the reciprocal nature of coupling between sensing and action, as well as between individuals and collectives.

Disclosures: **Y. Günzel:** None. **H. Kübler:** None. **E. Couzin-Fuchs:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.11

Topic: F.01. Neuroethology

Support: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy -EXC 2117 -422037984

Title: The visual mechanism of collective motion in desert locusts

Authors: ***C.-Y. LEE**[1,2,3], S. SAYIN[1,2], I. D. COUZIN[1,2,4], A. BAHL[1,2], E. COUZIN-FUCHS[1,2]; [1]Ctr. for the Advanced Study of Collective Behaviour, [2]Dept. of Biol., Univ. of Konstanz, Konstanz, Germany; [3]Intl. Max Planck Res. Sch. for Quantitative Behaviour, Ecology and Evolution from lab to field (IMPRS-QBEE), Konstanz, Germany; [4]Dept. of Collective Behaviour, Max Planck Inst. for Animal Behavior, Konstanz, Germany

Abstract: A central challenge in neuroscience is how the nervous system integrates information from the environment to generate appropriate behavioural outputs. It is increasingly acknowledged that sensory-motor transformations are inherently bidirectional and context-dependent, requiring experiments to be conducted under appropriately naturalistic conditions for the context in which the nervous system evolved. A prominent case in which the sensory-motor transformation loop is essential in order to explain behavior is the regulation of collective motion in group-living species, where individuals both impact, and are impacted by, the motion of others. Coordinated motion is exhibited by a diverse range of species and although it has been described by multiple abstract computational models, the sensory-motor pathways which regulate this behavior remain unclear. A ubiquitous feature to account for is individuals' capacity to detect and prevent collisions with others, a property that is visually-mediated in many species,

and depends on the relative motion of others in the visual field. Visual features of importance may include the size, position and/or rate of approach (loom) of other individuals. In a visual scene that contains multiple moving objects, it becomes less clear how salient features, such as the average direction of travel, thought to be a key feature in regulating collective motion, are extracted. To gain insight into what visually features are employed by organisms exhibiting collective behavior, we employ a custom-made immersive virtual reality technology, which allows decoupling stimulus and response, to examine principles underlying collective motion in a classic swarming system, the desert locust. Our preliminary results demonstrate that focal individuals to align their motion direction with the virtual group in which they are embedded. Based on that, we continue with dissecting the role of different visual features, such as insect density and motion coherence to both test classic models of collective motion, and inform new biologically-motivated models of swarm behaviour.

Disclosures: C. Lee: None. **S. Sayin:** None. **I.D. Couzin:** None. **A. Bahl:** None. **E. Couzin-Fuchs:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

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Program #/Poster #: 644.12

Topic: F.01. Neuroethology

Support: NIH U19NS104653
NIH 2R44OD024879
SCGB 542973

Title: Central regulation of autonomic and cardiac activity

Authors: *K. HERRERA[1], E. SONG[1], L. HERNANDEZ-NUNEZ[2], V. RUTTEN[4], M. B. AHRENS[5], F. ENGERT[3], M. C. FISHMAN[1];
[2]Dept. of Mol. and Cell. Biol., [3]MCB, [1]Harvard Univ., Cambridge, MA; [4]Janelia Farms, Ashburn, VA; [5]Janelia Res. Campus / HHMI, Ashburn, VA

Abstract: Much of neuroscience is focused on the role of the brain in generating adaptive motor commands based on external sensory stimuli. However, another crucial function of the brain is to govern the operation of the organs of the body and to optimize their action of current or predicted environmental and internal conditions. While the direct neural regulators of organ function in the autonomic nervous system (vagal and sympathetic) are known, little is understood about how the central nervous system orchestrates their activity. Here, we try to understand how the brain of the larval zebrafish integrates sensory information about threatening environmental changes to generate appropriate changes in heart rate. Using pharmacological perturbations, we find that the brain must be modulating cholinergic vagal activity. We then perform brain-wide imaging during threat presentation, where we observe that heart rate correlates with switches in

habenular functional states, as well as the activity of a glutamatergic nucleus within the thalamus that may be homologous to the periaqueductal grey. Optogenetic stimulation of this nucleus leads to abrupt reduction of cardiac activity, suggesting it is involved in upregulating vagal activity. Together our results are pushing toward a brain-wide description of a circuit responsible for transforming sensory input into changes in visceral dynamics.

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Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.13

Topic: F.01. Neuroethology

Support: NSERC Discovery Grant to JEL

Title: The impact of self-motion on electrosensory inputs in free-swimming weakly electric fish

Authors: *M. UPSHALL, A. R. SHIFMAN, J. E. LEWIS;
Biol. and Brain & Mind Inst., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Weakly electric fish emit an electric field to actively sense their environment. Objects with electrical properties different from that of water produce microvolt level perturbations in the field that are encoded by receptors distributed over the fish's skin. However, given the background noise of a complex sensory scene, it is not clear how fish use these tiny signals to capture their prey (*Daphnia magna*, 2-3mm). Furthermore, weakly electric fish swim omnidirectionally and constantly change their body shape, both of which produce additional and large perturbations in their electric field. One hypothesis suggests that this self-motion generates additional noise that must be cancelled out by downstream electrosensory pathways. However, the specific roles that such cancellation mechanisms play in dynamic free-swimming fish remains poorly understood. An alternative hypothesis suggests that fish actively use self-motion to improve information acquisition. Scanning (quick back and forth swimming) and tail bending are prominent during exploration and prey capture, yet how these behaviours contribute to the natural sensory inputs in freely swimming fish is unknown. So, does self-motion help or hinder electrosensory processing? As a first step, we characterize movement and electrosensory inputs in fish swimming in dynamic environments. We use pose-estimating software to report the fish's position in space and time, and then we compute the associated field perturbations using our recently developed software package, *fish2eod*. In this way, we quantify the detailed contributions of various movements to the natural electrosensory inputs during free swimming. Our results provide a quantitative context on which to base future studies on how self-motion impacts the responses of electrosensory pathways.

Disclosures: M. Upshall: None. **A.R. Shifman:** None. **J.E. Lewis:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.14

Topic: F.01. Neuroethology

Title: The serotonin type 2 receptors modulate acoustically-evoked escape behavior selection in zebrafish

Authors: J. DVORAK, I. RAY, M. CURRAN, R. OSBALDESTON, K. VILLAFAÑE, *R. A. JAIN;
Biol., Haverford Col., Haverford, PA

Abstract: In animals, system-wide or targeted neuromodulator activity in the nervous system contextually alter behavior. Serotonin (5-HT) is a neuromodulator that regulates decision-making and behavioral selection, with clinical relevance to neurological disorders such as ADHD and schizophrenia. Pharmacological manipulation of 5-HT is known to affect the acoustically-evoked behavior selection in zebrafish larvae, but it is not yet understood which of the diverse 5-HT receptors are responsible for this modulation. During an acoustic startle, zebrafish select between one of two stereotyped bouts: either a short-latency C-bend (SLC) or a long-latency C-bend (LLC), providing a robust model of ethologically relevant behavior selection in the nervous system. Here, we investigate the impact of 5-HT signaling in zebrafish startles using pharmacologic and genetic approaches. We hypothesized that serotonergic inputs into the startle circuit directly or indirectly modulate the acoustic startle behavior selection through restricted subtypes of 5-HT receptors. To test this, we screened an array of drugs affecting specific 5-HT receptor subtypes for candidates that acutely modulate the startle behavior. Five-day-old zebrafish were exposed to 0-500μM of each drug 30 minutes before testing, and they were delivered spaced acoustic stimuli of low, medium, and high intensity to observe shifts in behavior selection bias (n = 72-108 fish per condition). FLOTE software was used to track fish and identify behavioral responses from video recording data in an unbiased and observer-independent manner. Fish treated with the $5\text{-HT}_{2b/2c}$ receptor agonist VER-3323 demonstrated a dose-dependent shift in bias towards SLC behavior, while those treated with the $5\text{-HT}_{2b/2c}$ receptor antagonist SB 206553 demonstrated an opposing shift towards LLC behavior. This research suggests a previously uncharacterized role of 5-HT signaling through one or more 5-HT type 2 receptor subtypes in the zebrafish acoustic startle circuit and shows potential for further translational applications. To interrogate the genetic basis for startle behavior selection modulated by 5-HT, we have designed CRISPR guides to generate fish with individually mutant 5-HT_{2B} or 5-HT_{2C} receptor genes. Together, our pharmacological and genetic investigation of acoustic startle responses in zebrafish characterize a novel role of 5-HT in modulating behavioral selection.

Disclosures: **J. Dvorak:** None. **I. Ray:** None. **M. Curran:** None. **R. Osbaldeston:** None. **K. Villafañe:** None. **R.A. Jain:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

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Program #/Poster #: 644.15

Topic: F.01. Neuroethology

Support: ERC 852765
Wellcome Trust
Natural Sciences and Engineering Research Council of Canada

Title: Behavioural effects of claustrum silencing in mice

Authors: *D. K. OLIVER, T. HEATH-COLEMAN, A. M. PACKER;
Dept. of Physiology, Anatomy, and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: The claustrum is a long, thin, bilateral structuresequestered beneath the cortex. Extensive anatomical research has revealed thatthe claustrum has dense reciprocal connections with most cortical regions butsends relatively few projections to subcortical regions. Despite decades ofanatomical research into the exceptional connectivity of the claustrum, studiesof the claustrum's function have lagged behind. Numerous potential functionshave been hypothesized, but there is little consensus. Historically, lesionstudies in humans and animals have proven useful for localizing functionswithin the brain. However, the unique shape and inaccessible location of theclaustrum mean that specific lesions are rare. While a recent review of humanlesions linked the claustrum to perception, pain, sleep, and salience, studiesusing animal models are lacking. Previous studies have investigated theconsequences of claustrum inhibition, but no study has provided a comprehensiveassessment of the behavioural consequences of claustrum silencing. To addressthis gap, we bilaterally silenced the claustrum in male mice using viralexpression of the tetanus toxin light chain (TetTox) in a recently developedtransgenic line. After allowing time for viral expression, mice were thenexposed to a battery of ethologically motivated behavioural tests. Recordingsof these tests were scored by a blinded observer. When compared with sham,claustrum silencing had no significant effect on motor coordination, locomotorbehaviour, sociability, exploratory behaviour, anxiety, or sensorimotor gating(n=12 + 11). These results highlight the challenges involved in linking theclaustrum to behavioural outcomes, and call for further study into the functionof this enigmatic brain region.

Disclosures: D.K. Oliver: None. **T. Heath-Coleman:** None. **A.M. Packer:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.16

Topic: F.01. Neuroethology

Support: NIH BRAIN Initiative Grant U01NS108637

Title: Neural division of labor: the marine gastropod Berghia defends against attack using its PNS for rapid retaliation and its CNS to erect a defensive screen

Authors: J. W. BROWN[1], O. H. BERG[3], C. STOERCK[4], A. BOUTKO[2], W. N. FROST[1]; [1]Ctr. for Brain Function and Repair, [2]Chicago Med. Sch., Rosalind Franklin Univ., North Chicago, IL; [3]Lake Forest Col., Lake Forest Col., Lake Forest, IL; [4]Dept. of Psychology, California State University, Fullerton, Fullerton, CA

Abstract: Gastropod mollusks are often synonymous with slow movement, a reputation well earned by the repertoire of decidedly "sluggish" behaviors exhibited by many members of this taxon. Here, we characterize a dramatic, uncharacteristically rapid defense behavior, "bristling," in the aeolid nudibranch *Berghia stephanieae.* This behavior, which can be initiated in as little as 5 ms and climaxes in under 1 s following stimulation, involves the coordinated pivoting and/or elevation of the cerata, dozens of appendages emerging from the animal's dorsum whose tips concentrate nematocysts digested from *Berghia*'s anemone prey and thus deter prospective predators when directed at them. We elicited bristling through tactile stimulation by applying a Von Frey hair at three body loci—head, midbody, and caudal—in both intact and decerebrated animals, acquiring 200-fps video at high magnification through a stereoscope. Our investigations in intact animals revealed that bristling consisted of a stereotyped two-stage response: an initial adduction and wrapping of proximate cerata around the offending stimulus (Stage 1), followed by a coordinated radial extension and dispersion of remaining cerata to create a dramatic, pincushion-like defensive screen (Stage 2). The bristling response, which radiated from the stimulus site to ultimately recruit cerata along the length of body, was observed to propagate linearly in the posterior direction when the head was stimulated, while the recruitment of head and midbody cerata following caudal stimulation occurred simultaneously on average, suggesting nonlinear response propagation towards the head. In decerebrated specimens, Stage-1 bristling remained largely preserved, while the whole-body, defensive screen erected by intact animals in Stage-2 bristling was replaced by an uncoordinated, non-radial form of ceratal pivoting that spread to varying distances away from the induction site; this diminished propagative response was moreover significantly slowed in both the anterior and posterior directions relative to Stage-2 bristling in intact animals. Together, these observations implicate the peripheral and central nervous systems in primarily mediating Stages 1 and 2 of bristling, respectively, and suggest that the behavior propagates through the body utilizing both peripheral and central nerve networks supporting different signaling kinetics. The findings in this study provide insight into the cooperative patterning of a flamboyant defense behavior across two neural systems, the cellular correlates of which can be further explored in electrophysiological investigations of *Berghia*'s nervous system.

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Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.17

Topic: F.01. Neuroethology

Title: Species-appropriate, stimulating housing prevents compromising the well-being of laboratory mice without increasing variability.

Authors: L. LEWEJOHANN, P. MIESKE, U. HOBBIESIEFKEN, S. SIKDER, *K. DIEDERICH;
German Ctr. for the Protection of Lab. Animals (Bf3R), German Federal Inst. for Risk Assessment, Berlin, Germany

Abstract: Low-stimulus and restrictive housing can cause the development of a wide variety of behavioral disorders. These are considered indicators of impaired welfare and are associated with changes in the central nervous system. In scientific experiments, such pathological abnormalities can affect the experimental results. Therefore, good animal welfare is central to the reliability and thus the quality of animal experiments. However, the adoption of improved husbandry systems is biased by the concern that abandoning restrictive but fully controllable husbandry practices will lead to an increase in data variability.
Here, we investigated whether long-term housing in a conventional, restrictive housing system leads to behavioral abnormalities and neurological impairment compared to housing in an enriched cage environment as well as in a more species-appropriate housing in a semi-natural environment. Furthermore, we aim to understand how and to what extent variability in behavioral, morphological, physiological, and neuroanatomical data unfolds over time and whether variability is affected by housing conditions. Female C57Bl/6J mice were housed for 22 month in conventional cages (CON, n=12), enriched cages (ENR, n=12) and a semi-natural environment (SNE, n=20). We recorded the activity and spatial distribution of animals in the SNE by radio-frequency identification. Neurophysiological outcome measures included hippocampal volumetry, differential analysis of neuronal cell formation and differentiation using proliferation markers CldU and IdU, and morphometric analysis neurons in the hippocampus and amygdala. Focal animal observation revealed that CON mice showed significantly more inactive and stereotypic behavior than ENR mice. SNE mice showed no stereotypic behavior. These behavioral abnormalities were associated by distinct impairments in brain morphology and physiology. In SNE animals, we detected an increase of diversification in roaming behavior over time with stabilizing activity patterns at the individual level. This suggests that individual differences do indeed emerge and stabilize over time. Most interestingly, the variability of data from animals from the ENR and SNE systems did not exceed that of animals from the conventional systems, nor did it exceed literature data obtained from mice living under conventional laboratory conditions.
We conclude that species-appropriate housing of laboratory animals is crucial to prevent the emergence of behavioral disorders and neurological impairments. Furthermore, improving

animal welfare through changes in husbandry conditions do not lead to a deterioration in data quality.

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Poster

644. Neuroethology: Sensory Motor Systems I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.18

Topic: F.01. Neuroethology

Title: Modulation of digastric reflex responses during various situations in conscious rats

Authors: *T. CHOTIRUNGSAN[1,2], J. MAGARA[1], T. TSUJIMURA[1], M. INOUE[1];
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Abstract: Introduction: Digastric muscle (Dig) is composed of two parts, which are anterior belly (AntDig) and posterior belly (PostDig) innervated by different motor neurons. AntDig reflex is known to be jaw-opening reflex and numerous studies have reported modulation of anterior digastric reflex responses in functions. However, the PostDig reflex has never been investigated before. This is the first time to reveal the nature and modulation of PostDig reflex during resting, chewing, licking, and swallowing. We expected that the modulation pattern in PostDig differs from that in AntDig.
Methods: In this study, Sprague Dawley rats were chronically implanted electrodes for recording electromyogram (EMG). Via the head connector and cables, the rat could freely behave in the recording session. During resting, chewing, licking, and swallowing periods of conscious rats, both the AntDig and PostDig reflexes were evoked by low-threshold electrical stimulation of the inferior alveolar nerve. EMG burst of thyrohyoid, masseter, and both Dig muscles were used to identify swallowing, jaw-closing, and jaw-opening phases, respectively. In each situation, digastric reflex responses in PostDig were compared with that in AntDig.
Results: There was no difference in the threshold and latency between AntDig and PostDig reflex responses. the results suggest that PostDig may also be a disynaptic reflex evoked by non-noxious trigeminal stimulation. Both reflex responses were significantly inhibited during feeding period compared with resting period.
Conclusions: These results suggest that PostDig coordinated with AntDig to serve not only orofacial but also laryngeal protective mechanisms. We predict that both reflexes should be reduced during ingestion, especially in the jaw-closing phase, to prevent unnecessary occurrences.

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Poster

644. Neuroethology: Sensory Motor Systems I

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 644.19

Topic: F.01. Neuroethology

Support: ERC

Title: Song duels adhere to context-dependent rules in nightingales

Authors: *G. COSTALUNGA, D. VALLENTIN;
Max Planck Inst., munich, Germany

Abstract: Adapting vocal outputs depending on contexts is an essential feature of interactive vocal exchanges, like complex human conversations. We investigated the context-dependent flexibility of nightingales, birds with a repertoire of up to 200 songs which perform sophisticated song duels against conspecifics. To test whether song performance is depending on the social context, we exposed wild nightingales to different playbacks. We found that both temporal and spectral aspects of song duels were adjusted depending on the playback familiarity. Specifically, nightingales reduced song duration, increased pauses and reduced the amount of overlaps with playbacks when singing against the bird's own songs (BOS). Using a deep learning approach, we found that playbacks elicited song-matching, during which the bird responded with the same song it just heard. This behavior was increased in response to BOS indicating that familiar songs have the potential to trigger a specific vocal response. During the BOS presentation the birds also performed song anticipations during which they predicted the next song of the playback. This suggests that nightingales sing a predefined sequence of songs which can be evoked by external auditory input. Next, we wanted to study the neural substrates of song-matching behavior by performing intracellular recordings in the premotor HVC of hand raised and song-tutored nightingales while exposing them to song playbacks of different familiarity. HVC neurons selectively responded to the presentation of songs of the bird's repertoire. Taken together, these results show nightingales' ability to adjust vocal outputs depending on the context, and the presence of precise auditory integration necessary for song-matching in premotor neurons of the nightingale's brain.

Disclosures: **G. Costalunga:** None. **D. Vallentin:** None.

Poster

644. Neuroethology: Sensory Motor Systems I

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Topic: F.01. Neuroethology

Support: NSF Grant IOOS-1147172
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Title: Grasshoppers and goldfish use different neural computations to produce similar visually-guided escape behavior in response to impending collision.

Authors: *M. EISENBRANDT[1], R. B. DEWELL[1], T. L. CARROLL-MIKHAIL[2], T. PREUSS[2], F. GABBIANI[1];
[1]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [2]Psychology, City Univ. of New York, Hunter Col., New York, NY

Abstract: Collison avoidance and escape behaviors are vital for survival and animals often have neurons tuned for collision detection. These neurons are most sensitive to objects approaching at a constant velocity, which is modeled using two-dimensional simulated equivalents (‘looming stimuli’). η-type neurons have peak responses a set delay after the simulated object reaches a threshold angular size and their activity is described by the product of the stimulus’ angular speed and the negative exponential of its angular size. One η-type neuron is the lobula giant movement detector (LGMD) in grasshoppers which synapses to the descending contralateral movement detector (DCMD) neuron whose spikes are in 1:1 correspondence. The Mauthner cell in goldfish is a looming sensitive κ-type neuron, described by the product of the angular size of the stimulus with a negative exponential of its angular size. Both models predict similar responses to approaches with constant velocity but different responses for accelerating or decelerating approaches. Our group used grasshoppers and goldfish to assess response timings to looms of constant angular or linear velocities in free behaving fish and grasshoppers, and the DCMD responses of grasshoppers. Due to different hunting behaviors of predators, understanding how changes in acceleration impact response can help us better understand escape behaviors and characterize the neural circuitry involved in calculating and executing these escapes.

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Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 645.01

Topic: F.01. Neuroethology

Support: Midwestern University Intramural Support

Title: Corticotropin Releasing Factor (CRF)-family expression in the zebra finch brain

Authors: S. PATEL, ***C. OLSON**;
Midwestern Univ., Glendale, AZ

Abstract: Corticotropin releasing factors (CRFs) are closely related signaling peptides with roles in the response to stressors. Due to effects of CRF signaling on songbird vocal learning, we characterize the gene family and their receptors in the zebra finch and by *in situ* hybridization show expression patterns for select CRFs and their receptors. The zebra finch genome, like non-mammalian amniotes, contains all five CRFs (CRH1, CRH2, UCN, UCN2, UCN3), two CRF receptors (CRHR1 and CRHR2) and a binding protein (CRHBP). CRH2 is absent in mammals and some fish lineages, thus understanding its expression in the finch brain will provide insights into how stress in managed in the brain. CHR1 (CRH) is the trophic hormone at the top of the hypothalamic-adrenal axis and its expression in the paraventricular nucleus is marked. In addition, there exists bilateral expression in other hypothalamic and midbrain structures, including expression in the locus coeruleus. Strong CRH1 expression occurs sparsely through most of the forebrain with elevated expression and cell density in the shell of the dorsolateral corticoid area. CRH1 lacks differential expression in vocal nuclei. In contrast, CRH2 is much limited in its expression, occurring in the hypothalamus and thalamus, and in large cells located adjacent to the Edinger-Westphal nucleus, suggesting functional overlap with the centrally-projecting UCN-expressing cells that occur in mammals. CRH2 expression is absent in the zebra finch forebrain. To date we lack evidence of UCN expression in the finch brain. CRHBP shows limited expression in pontine and midbrain structures with sparse expression throughout the forebrain and particularly prominent in large cells in vocal nucleus HVC. CRHR1 is the primary receptor for CRH1, CRH2 and UCN, thus we limit our description to this receptor. CRHR1 has limited expression in hypothalamic and midbrain structures. The forebrain expression is elevated in the hippocampus, entopallium and the arcopallium. It is a positive marker of the corticoid nucleus and auditory field L; it is a negative marker of the song nucleus RA. Follow up coexpression analysis provides additional insights on the functional nature of CRF signaling and in how stress may modulate learning in the songbird model.

Disclosures: S. Patel: None. **C. Olson:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 645.02

Topic: F.01. Neuroethology

Support: CODI-UdeA 2020-33794

Title: Sonic Hedgehog pathway regulation in Hippocampus and HVC during song stimulation in Serinus canaria

Authors: *R. POSADA-DUQUE[1], H. RIVERA-GUTIERREZ[2], M. BERMUDEZ-MUÑOZ[3], A. DAZA-ZAPATA[1];
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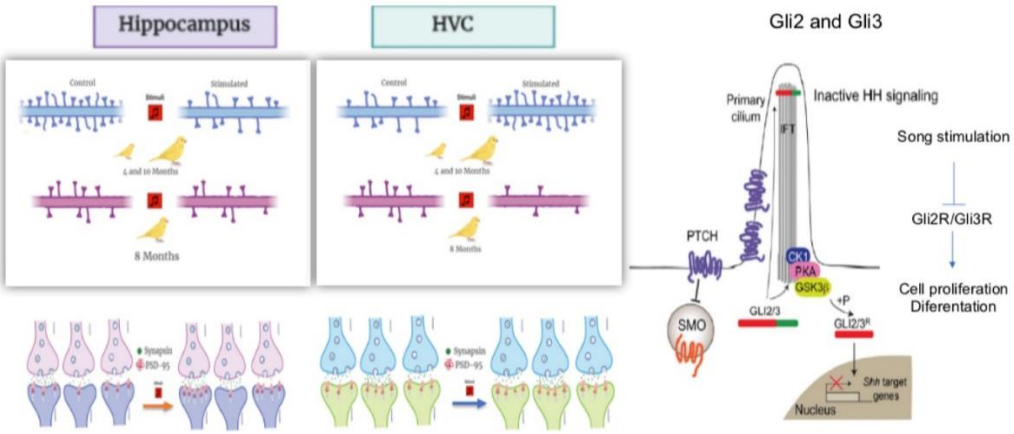
Abstract: Serinus canaria are open-ended learners, they can learn and add new songs to their repertoire throughout their lives. Learning, memorizing and producing a song, requires interconnected neuronal structures in the avian brain. Despite the vast amount of knowledge about the roles of specific brain structures in song production and learning, little is known about how song stimulation generates changes of dendritic spines, synapses and cell signaling in birdsong brains. In this study, we have analyzed the expression of proteins related with dendritic spines (MAP2, F-actin), with synapses (PSD95, synapsin) and with SHH cell signaling (SHH, GLI1, GLI2, GLI3), in the brains of serinus canaria at different stages of their song learning process (4, 8 and 10 months after hatching), upon conspecific song stimulation. We found that protrusions of F-actin and average spots of synapsin and PSD95 are similar in individuals aged 4 and 10 months but differs in individuals of 8 months old, suggesting that song could be crystallized at 8 months after hatching in canaries raised in an animal facility in the tropics. Moreover, we found that while the ligand SHH is expressed in the ventral pallidum, the cells that express the SHH-response transcription factors are located in different brain nuclei: GLI1 is expressed in the hyperpallium, GLI2 and GLI3 are found in the hippocampus and HVC. Interestingly, when GLI1 expression increases with song stimulation in 4 and 10 months-old individuals, GLI2 and GLI3 expression decreases, pointing to a SHH/GLI1 signaling activation upon song stimulation during sensitive song learning periods. Taken together, these results indicate that SHH/GLI signaling pathway activation changes upon song stimulation in serinus canaria during their song learning and memory phase, which is associated with modifications in dendritic spines and synapses in their hippocampus and HVC.



Disclosures: **R. Posada-Duque:** None. **H. Rivera-Gutierrez:** None. **M. Bermudez-Muñoz:** None. **A. Daza-Zapata:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 645.03

Topic: F.01. Neuroethology

Support: NIH Grant DC004274
NIH Grant GM089700-01

Title: Betz cells in primate cortex and vocal cortical motor neurons in song birds express Kv3 channels and exhibit ultra-narrow spikes for high frequency firing

Authors: *B. ZEMEL[1], A. NEVUE[3], A. DAGOSTIN[2], L. E. TAVARES[5], P. V. LOVELL[6], D. Z. JIN[7], C. V. MELLO[6], H. P. VON GERSDORFF[4];
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Abstract: The speed and accuracy of fine motor skills depend on the spiking activity of upper motor neurons. In mammals, subclasses of these neurons contain unique excitable features that may enable the temporal precision required for these skills. Zebra finches are excellent models for testing how upper motor neurons provide the temporal precision required for rapid, precise behaviors. Their songs contain multiple, acoustically complex syllables, produced on sub-second time scales, and are controlled by pallial nuclei that form microcircuits analogous to those in the layered mammalian neocortex. Brainstem projecting vocal-motor neurons of the robust nucleus of the arcopallium (RAPNs) are spatially separated from upper-motor neurons of the dorsal intermediate arcopallium (AId neurons), a region implicated in non-vocal somatic motor functions. Consistent with the motor theory of vocal learning origin, RA and AId have similar molecular profiles, suggesting that RA may have evolved as a specialization of AId. Here we provide evidence linking the expression of the Kv3.1 channel to the ultra-narrow RAPN spike waveform. In patch clamp recordings within brain slices from adult male finches, RAPNs exhibited spikes with narrower half-widths and larger maximum repolarization rates than AId neurons. Spike waveforms from RAPNs were more sensitive than AId neurons to pharmacological compounds targeting Kv3.1 channels. Additionally, *in situ* hybridization revealed that Kv3.1 mRNA is more highly expressed in RA compared to AId, whereas other Kv3 family genes are not differentially expressed between RA and AId. We suggest that Kv3.1 regulates the intrinsic excitability of RAPNs on a sub-millisecond time scale, making them specialized control units for the production of complex vocalizations in the zebra finch. These specializations of RAPNs resemble those of pyramidal Betz cells in layer 5 of primate motor cortex, which also exhibit ultra-narrow AP half-widths and differentially express Kv3.1, whereas similar cells are absent in rodents. This may reflect a convergent evolution of upper motor neuron properties in specific mammalian and avian species that enables precise spike firing at high frequencies. Our findings provide insight into the regulation of excitability in specialized cortical circuits of primates and songbirds that are involved in fine motor control.

Disclosures: B. Zemel: None. **A. Nevue:** None. **A. Dagostin:** None. **L.E. Tavares:** None. **P.V. Lovell:** None. **D.Z. Jin:** None. **C.V. Mello:** None. **H.P. von Gersdorff:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 645.04

Topic: F.01. Neuroethology

Support: CONACYT Grant #337259

Title: Assessing the ZENK induction in the auditory forebrain of a Neotropical sparrow as Neural response to species-specific songs

Authors: *R. A. FERNÁNDEZ-GÓMEZ[1,2], D. HERNÁNDEZ-BALTAZAR[2,3], S. K. MISCHLER[4], C. B. STURDY[4], J. E. MORALES-MÁVIL[2], J. SOSA-LÓPEZ[5,3];

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Abstract: With a defined neural circuit, along with great acoustic variability and complexity, birdsong is an excellent trait to elucidate questions about the neural basis of birds' acoustic communication. A well-studied immediate early gene (IEG), ZENK (Egr-1) has been widely used as marker to test the neural activation in the forebrain areas involved in the auditory processing and acoustic production. However, most studies have been focused on assessing the dynamic of neural activation in high processing auditory areas in species with marked vocal divergence where the recognition abilities of intraspecific signals play an important role in speciation processes. Here, we assessed neural activity in Olive sparrows (*Arremonops rufivirgatus*) in response to divergent acoustic stimuli. We played back simulated songs of local and foreign intruders (i.e., geographic variation) to sparrows and assessed neural activity by counting ZENK immunoreactive cells [ZENK (+)] in two auditory forebrain areas (the caudomedial mesopallium, CMM; and the caudomedial nidopallium, NCM). In this study, the immunohistochemical staining method was previously validated. We found no significant differences in the amount of ZENK (+) cells among treatments; however, we found a large effect size where there was a large increase in ZENK induction to foreign songs. Our results are consistent with previous studies, supporting the hypothesis that the neural activity in auditory forebrain areas may be fostered by specific acoustic features in the auditory stimuli, specifically song.

Disclosures: R.A. Fernández-Gómez: None. **D. Hernández-Baltazar:** None. **S.K. Mischler:** None. **C.B. Sturdy:** None. **J.E. Morales-Mávil:** None. **J. Sosa-López:** None.

Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.05

Topic: F.01. Neuroethology

Support: European Union's Horizon 2020 Marie Skłodowska-Curie grant 101025762 to MDR

Title: Combining expansion light sheet microscopy (ExLSM) and in vivo two photon imaging to resolve HVC-X projection neuron subclasses

Authors: *M. D. ROCHA[1], S. MA[2], R. HAHNLOSER[1], F. DITTRICH[2], M. GAHR[2], D. N. DÜRING[1];

[1]INI, ETH Zurich, Zurich, Switzerland; [2]Max-Planck-Institute for Biol. intelligence, Seewiesen, Germany

Abstract: The brains of songbirds have deeply interested researchers for many decades. Neuroscientists' interest has been prompted by the songbird brain's distinctive neural organisation and striking plasticity, as well as the cognitively complex behaviours it encodes. In particular, songbirds' astonishing vocal learning abilities, the skill to produce vocalisations to imitate heard sounds that also gives rise to human speech, has made their brain circuits the spotlight of many avenues of investigation. Such previous studies on the song system, the group of interconnected brain nuclei responsible for vocal learning and production in songbirds, have provided a plethora of information on the variety of connections linking these nuclei, as well as their functional significance. Nonetheless, the projection neuron classes within this circuit have traditionally been separated merely on the basis of their anatomical location and projection targets. This is particularly astonishing given that evidence has been provided to justify further subdivisions, including findings of distinct morphological, functional, and molecular subclasses. Specifically, HVC-X neurons, which connect nucleus HVC (proper name), the songbird vocal premotor cortex analogue, to area X of the striatum, are usually treated as a homogeneous group, although clear evidence for at least four morphologically distinct classes has been available since the 1990s. This is striking given that these 1990s studies only analysed the morphology of a couple of dozen of the estimated tens of thousands of HVC-X neurons found in each hemisphere. Furthermore, nothing is known on the potentially specific functional roles of such HVC-X subclasses. To overcome this knowledge gap, we analyse the detailed morphology and neuronal activity of large numbers of HVC-X neurons in two songbird species, zebra finches and canaries. To achieve this, we take advantage of recent developments in viral vectors to specifically target songbird projection neuron populations and in expansion and light sheet microscopy (ExLSM) to enable high resolution imaging of HVC-X neurons across the large volume of whole HVCs. In addition, we connect findings on morphological subclasses to their potential functional roles by examining their activity during singing using *in vivo* two photon calcium activity imaging.

Disclosures: **M.D. Rocha:** None. **S. Ma:** None. **R. Hahnloser:** None. **F. Dittrich:** None. **M. Gahr:** None. **D.N. Düring:** None.

Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.06

Topic: F.01. Neuroethology

Support: NIH 1R56NS094831
NIH 1UF1NS115821-01

Title: Low-threshold and calcium-dependent potassium currents regulate the intrinsic firing properties of forebrain-projecting HVC_{RA} neurons in zebra finches

Authors: *A. DAOU[1,2], S. CHOKER[1], D. MARGOLIASH[2];
[1]American Univ. of Beirut, Beirut, Lebanon; [2]Univ. of Chicago, Chicago, IL

Abstract: The telencephalic nucleus HVC within the songbird, analogue to the mammalian pre-motor cortex, produces stereotyped instructions through the motor pathway leading to precise, learned vocalization. The HVC contains multiple neural populations, including neurons that project to the RA (robust nucleus of arcopallium), to Area X (of the avian basal ganglia), to Avalanche (higher order auditory structure), and interneurons. These three populations are interconnected with specific patterns of excitatory and inhibitory connectivity, and they fire with characteristic patterns both *in vivo* and *in vitro*. Premotor HVC_{RA} neurons in particular play a critical role in orchestrating the neural circuitry that guides the bird's song production. We performed whole cell current-clamp recordings on zebra finch HVC_{RA} neurons within brain slices to examine their intrinsic firing properties and determine which ionic currents are responsible for their characteristic firing patterns. We show that these neurons exhibit a diversity in their firing activity when stimulated with current pulses in slices ranging from transient to stuttering patterns, classifying them into three subtypes. We developed conductance-based models for the different neurons in each subtype and calibrated the models using data from our brain slice work, yielding mechanistic descriptions of how the interplay of ion currents give rise to the response properties of each neuronal class. These predictions were then tested and verified in the slice using pharmacological manipulations. The model and the pharmacology highlighted important roles for the low-threshold potassium currents (the D-type Kv1 channel) and (the M-type Kv7 channel) as well as the Ca^{2+}-dependent K^+ current in driving the characteristic neural patterns observed in HVC_{RA}. The data suggest that the intrinsic properties for one of the HVC_{RA} subtypes exhibit a within-bird homogeneity and across-birds heterogeneity; albeit not as strong segregation as we previously showed for the X-projecting neurons. These results open a host of related experiments we are pursuing that can yield additional insights into the mechanisms of song production.

Disclosures: A. Daou: None. **S. Choker:** None. **D. Margoliash:** None.

Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.07

Topic: F.01. Neuroethology

Support: 5R01DC012938-08

Title: Morphological reconstruction of RA projection neurons and AId neurons from the zebra finch

Authors: *L. E. S. TAVARES[1], B. M. ZEMEL[2], A. A. NEVUE[3], A. DAGOSTIN[2], P. V. LOVELL[3], C. V. MELLO[3], H. VON GERSDORFF[2], D. Z. JIN[1];

[1]Dept. of Physics, Penn State Univ., University Park, PA; [2]Vollum Inst., [3]Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: The robust nucleus of the arcopallium (RA) is essential for song production in songbirds. Projection neurons in the RA (RAPNs) are thought to be important in the control of the vocal output. However, no study has previously attempted a full 3D reconstruction of these neurons. Here we use ShuTu [1] to reconstruct the morphology of biocytin-filled RAPNs from adult zebra finches. Using our reconstructions, we extract geometrical properties and quantify dendritic spines in RAPNs. We then contrast our RAPN reconstructions to those of neurons in the adjacent dorsal intermediate arcopallium (AId), an area involved in somatic motor control. We find that RAPNs exhibit larger spines overall but significantly lower spine densities when compared to AId neurons. For all neurons, we also observe that spine density along the dendrite is low near the cell body, peaks midway and decreases near the tip. When corrected for anisotropic shrinkage artifacts due to tissue fixation [2], our reconstructions predict capacitance values close to those found in electrophysiological measurements using whole-cell patch clamp [3]. Finally, we estimate surface-to-volume ratios and find relatively large values for both RAPNs and AId neurons, suggesting high energetic demand. Indeed, RAPNs fire spikes at high frequencies in adult male finches and the RA nucleus shows strong staining for cytochrome oxidase, a mitochondrial protein correlated with ATP production and cellular metabolism [4]. Overall, our work contributes to the characterization of RAPNs and AId neurons in the zebra finch and provides new tools for morphological analysis.
[1] Jin, D. Z., Zhao, T., Hunt, D. L., Tillage, R. P., Hsu, C. L., & Spruston, N. (2019). ShuTu: Open-Source Software for Efficient and Accurate Reconstruction of Dendritic Morphology. Frontiers in Neuroinformatics, 13(October), 1-19.
[2] Pyapali, G. K., Sik, A., Penttonen, M., Buzsaki, G., & Turner, D. A. (1998). Dendritic properties of hippocampal CA1 pyramidal neurons in the rat: Intracellular staining in vivo and in vitro. Journal of Comparative Neurology, 391(3), 335-352.
[3] Zemel, B. M., Nevue, A. A., Dagostin, A., Lovell, P. V., Mello, C. V., & von Gersdorff, H. (2021). Resurgent Na+ currents promote ultrafast spiking in projection neurons that drive fine motor control. Nature Communications, 12(1), 1-23.
[4] Adret, P., & Margoliash, D. (2002). Metabolic and neural activity in the song system nucleus robustus archistriatalis: Effect of age and gender. Journal of Comparative Neurology, 454(4), 409-423.

Disclosures: L.E.S. Tavares: None. **B.M. Zemel:** None. **A.A. Nevue:** None. **A. Dagostin:** None. **P.V. Lovell:** None. **C.V. Mello:** None. **H. von Gersdorff:** None. **D.Z. Jin:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 645.08

Topic: F.01. Neuroethology

Support: NIH RO1 MH070712

Title: Human speech-related genes are direct targets of zebra finch FoxP2 across developmental and behavioral conditions

Authors: G. L. GEDMAN[1], D. FACTOR[2], G. VOJTOVA[2], T. KIMBALL[2], *S. A. WHITE[1]; [1]Integrative Biol. and Physiol., [2]UCLA, Los Angeles, CA

Abstract: Vocal learning is a rare, convergent behavior underlying song and speech production in songbirds and humans, respectively. The FOXP2 transcription factor is critical for this behavior, as disrupting FOXP2 function leads to impairments in proper song and speech learning across species. In songbirds, FOXP2 exhibits dynamic expression levels during singing, suggesting that identification of the subsequent regulation of downstream target genes can offer important insights into the molecular mechanisms of vocal learning. However, these target genes are currently unknown, as well as how they change across developmental and behavioral conditions. We conducted a ChIP-Seq experiment to identify FOXP2 binding sites in the zebra finch (*Taeniopygia guttata*) across sex (adult male/female), development (juvenile male/female), and behavior (juvenile male singing/quiescent) conditions. We found robust FOXP2 binding sites in all conditions, mostly (~75%) concentrated in gene promoter regions. However, the total number of genes with FOXP2 promoter binding varied greatly across conditions (207 – 812), suggesting specialized roles in development and behavior. We conducted gene ontology analyses on these putative FOXP2 target genes and found robust enrichment for human speech and language related functions in males only, consistent with zebra finch male-specific singing behavior. We found FOXP2 regulates fewer speech related genes in quiescent juvenile males relative to silent adult males, suggesting the expansion of this gene regulatory network with development. We found the fewest speech-related gene regulation in juvenile males following singing, consistent with FOXP2 downregulation as a result of this behavior. The ZEB2 transcription factor, which is also associated with speech deficits in humans when mutated, showed robust FOXP2 binding in adult males. Immunohistochemical data from both genes showed reciprocal patterns of expression, suggesting FOXP2 is serving a repressive role in this context. Overall, we provide the first categorization of the regulatory landscape of FOXP2 in the zebra finch across a variety of contexts, offering hundreds of potential target genes for future study. This work promises to provide important insights into the molecular underpinnings of vocal learning function and evolution across species.

Disclosures: G.L. Gedman: None. **D. Factor:** None. **G. Vojtova:** None. **T. Kimball:** None. **S.A. White:** None.

Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.09

Topic: F.01. Neuroethology

Support: NSF Grant 1633516

Title: In vivo imaging in transgenic songbirds reveals superdiffusive neuron migration in the adult brain

Authors: *N. R. SHVEDOV[1], T. J. GARDNER[2], B. B. SCOTT[1];
[1]Boston Univ., Boston, MA; [2]Univ. of Oregon, Eugene, OR

Abstract: Neuron migration is widespread throughout the adult songbird forebrain, where newly-born neurons are added to circuits that contribute to vocal learning. Evidence for radial migration along glial scaffolds has been observed histologically, in cultured explants, and with in vivo imaging. In addition, data from time-lapse in vivo imaging studies have suggested the possibility of a second form of migration, termed "wandering" migration, in which cells are not associated with radial glia and instead follow tortuous paths. In vivo imaging has high potential to elucidate the dynamics of neuroblast migration, however, previous labeling strategies yielded limited numbers of migratory cells and precluded detailed quantitative analysis. Here, we leverage transgenic, GFP+ zebra finches to systematically characterize migratory dynamics of motile cell populations across different ages and brain regions in vivo. Through histological analysis (using antibodies against NeuN, Hu, and doublecortin) we demonstrate that previously created transgenic zebra finches (Agate et al. 2009) exhibit GFP in the neurogenic lineage. With two-photon microscopy (2PM) through cranial optical implants, we found that GFP expression was sparse enough to detect cells with migratory morphology in vivo. By taking volumetric time-lapses, we followed over 370 migrating cells in unanesthetized juveniles and adults. We were able to track individual cells at regular intervals up to 12 hours. Cells in the juvenile HVC displayed high tortuosity and speed, consistent with previous in vivo studies. This migration pattern was observed not just in the male juvenile HVC but also in the pallium above the ventricular zone, adult male HVC, and the adult female nidopallium. Importantly, we found that this migration pattern was better fit by a superdiffusive model than a random walk or ballistic model. Moreover, by fitting this model to the trajectories of individual cells, we find that migration patterns appear to arise from a unimodal distribution. Together, these results suggest a single population of neuroblasts that undergo a spectrum of directed migration rather than passive diffusion and demonstrate that songbirds provide a framework for future studies into the mechanisms of adult-born neuron migration.

Disclosures: **N.R. Shvedov:** None. **T.J. Gardner:** None. **B.B. Scott:** None.

Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.10

Topic: F.01. Neuroethology

Support: R56 NS110951

Title: Exploration of a song responsive microRNA throughout development in juvenile zebra finches.

Authors: *R. BUTLER[1], S. E. LONDON[2];
[2]Dept of Psychology, [1]Univ. of Chicago, Chicago, IL

Abstract: Epigenetic mechanisms may be particularly informative for understanding how the accumulation of experience can promote and limit the ability to learn because they enable cells to transiently activate or repress transcription, a key component of long-term stable memory formation. Noncoding RNAs such as microRNAs (miR) are epigenetic regulators. miR regulate sex-specific biological and behavioral phenotypes and are differentially expressed throughout development. Thus, miR are well positioned to play a role in sex-specific, age-dependent learning processes.
miRs have been implicated in sensory song learning in both juvenile and adult zebra finch songbirds. In the auditory forebrain, a brain region required for sensory song learning, hearing song can change miR abundance in adult and juvenile males, and in adult males compared to adult females. In adults, one miR transcript, miR-2954, produces two mature miR, miR-2954-3p (miR-3p) and miR-2954-5p (miR-5p), which show distinct sexually dimorphic patterns in relative abundance after hearing song. This suggests that hearing song results in the translational availability of a unique set of RNAs in adult males compared to adult females that could be contributing to adult sensory song learning.
Both juvenile male and female zebra finches learn song. Juvenile males learn a song that reflects a sensory memory of a "tutor's" song they formed between post-hatch day 30 (P30)-P65. Females cannot sing, but behave in ways as adults that demonstrate they also form sensory song memories during this same period of development. This prompts the question: can miR-3p and miR-5p, song responsive sexually dimorphic miRs in adults, provide insight into whether or not juvenile females are doing the same kind of sensory song learning as juvenile males?
To answer this question, we used *in situ* hybridization to quantify the abundance of miR-3p and miR-5p within the auditory forebrain of males and females, after hearing song playbacks or being left in silence. We collected juveniles during the learning phase (P30 and P60), as well as before (P23) and after (P67) that phase. I predicted that hearing song would decrease miR-3p and miR-5p abundance in juveniles that are actively learning song, P30 and P60 males, but not P23 or P67 males or females at any age, and that this decrease would only occur in higher-order sensory integration sub-regions of the auditory forebrain, rather than in primary auditory cortex. If this prediction is supported, it would suggest juvenile females have a different way of processing song than juvenile males, which may indicate an additional sex difference in juvenile sensory song learning.

Disclosures: R. Butler: None. **S.E. London:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

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Program #/Poster #: 645.11

Topic: F.01. Neuroethology

Support: UVA Start-Up

Title: Seasonal plasticity of astrocytes in a sensorimotor nucleus controlling singing behavior and their possible role for re-establishment of homeostasis for future plasticity

Authors: W. C. TUCKER[1], J. BOYD[2], S. L. SHEPARD[2], ***T. A. LARSON**[2];
[2]Biol., [1]Univ. of Virginia, Charlottesville, VA

Abstract: The birth and functional incorporation of new cells, primarily neurons and astrocytes, into neural circuits in the adult vertebrate central nervous system is a fundamental process of neural plasticity. Given the relationship between neural plasticity and nervous system homeostasis and the maintenance of behavior, it is crucial to understand the functional relationships between plastic cell types and the mechanisms guiding their behavior. In the sensorimotor nucleus, called HVC of the song production circuit in Gambel's white crowned sparrow (*Zonotrichia leucophrys gambelli*), extreme plasticity in neuronal number coincides with quality of singing behavior. We find that supporting the breeding season increase in HVC neuron number and subsequent decrease in number during transition back to nonbreeding season, astrocytes scale in number within and across seasons. Interestingly, we find that astrocytes decrease in number delayed in time from the decrease in neuronal number and to levels lower than final homeostatic levels in stable nonbreeding season. These results suggest that astrocytes might participate in the clearance of dead neurons and then themselves die to allow new astrocytes to repopulate HVC. To elucidate whether or not previously described reactive proliferation following neuronal death in HVC generates astroctyes that restore nonbreeding season homeostasis, we performed a lineage trace study of the progeny from nearby proliferating neural progenitor cells. Identification of addition of newly-born astrocytes into HVC upon transition into nonbreeding season following neuronal death would lay the foundation for studies examining the role of astrocytes in restoring homeostasis and future re-growth of HVC in subsequent breeding seasons.

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Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.12

Topic: F.01. Neuroethology

Support: NIH 2R01NS099288
NIH F32DC018507

Title: The effects of dopamine and tutoring on the HVC microcircuit

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Abstract: Learning by imitation is central to speech, language, and many other important behaviors. Juvenile male zebra finches selectively memorize and copy songs produced by a conspecific adult tutor, which involves a learning mechanism that integrates social and auditory cues provided by a singing tutor. The forebrain sensorimotor nucleus HVC is a crucial site where auditory and social cues are integrated to drive the early stages of song learning. HVC receives auditory input from the nucleus NIf and dopamine (DA) input from midbrain (A11) neurons, the latter of which are activated by the presence of a live, singing tutor but not by a silent tutor or tutor song playback alone. We first sought to understand the physiological effects of DA, which we hypothesize signals the presence of a suitable vocal model, on various cells and synapses in HVC. We made whole cell patch clamp recordings from identified striatal-projecting (HVC_X) cells, premotor (HVC_{RA}) cells, and interneurons (HVC_{INT}) in brain slices prepared from untutored 45-55 day old juvenile males. Optogenetically stimulating NIf terminals elicited di- and poly-synaptic EPSCs that were affected in various ways by bath-applying DA. In most HVC_{INT}, bath applying DA reduced the latency to EPSC onset, suggesting that DA increases the excitability of excitatory cells in HVC that synaptically link NIf to these interneurons. Indeed, bath application of DA increased the intrinsic excitability and spontaneous EPSC frequency in multiple HVC cell types. Additionally, some HVC_X, HVC_{RA} and HVC_{INT} showed an increase in EPSC charge transfer following DA application, which may point to synaptic plasticity mechanisms within the local HVC circuit. We next sought to further assess how tutoring affects HVC neurons and their auditory inputs from NIf. In vivo fiber photometric measurements of calcium signals in specific HVC cell types revealed that tutoring rapidly increased the amplitude of singing-related calcium signals in both HVC_X and HVC_{RA} cells. Lastly, fiber photometric monitoring of NIf terminals in the juvenile's HVC showed that they were activated during singing, playback of tutor song, and encounters with a singing tutor, but that the amplitude of these signals remained the same before and after tutoring. These approaches highlight the broad and potent effects of DA on HVC cells and synapses while constraining the locus of tutor-driven effects to postsynaptic sites within the HVC microcircuit.

Disclosures: A.A. Mercer: None. **R.D. Mooney:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

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Topic: F.01. Neuroethology

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Title: Singing context-dependent activation of oxytocin receptor neurons in the avian basal ganglia

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Abstract: Although social behavior is typically necessary for organismal survival, the brain mechanisms that regulate decision-making in social contexts are not well understood. The vertebrate social behavior network has been implicated in regulating decision-making in social contexts and is reciprocally connected to the hypothalamic paraventricular nucleus (PVN), a major production site of oxytocin. Here we investigate the possibility that oxytocin signaling and social behavior network convey social contextual information to the songbird vocal control network. Using adult male zebra finches performing either social female-directed or non-social undirected song and fluorescence in situ hybridization, we quantified cellular co-expression of *EGR1* and *OTR* in the vocal control network. Males sang spontaneously for 45 minutes in the morning shortly after the start of their light cycle. As expected, we found *EGR1* highly expressed within the forebrain regions of the vocal control network, including Area X, in a non-social undirected singing context. Furthermore, *EGR1* expression in Area X was significantly decreased in a social female directed singing context. The percentage of *EGR1+* cells that were *OTR+* in non-social undirected singing was comparable to that observed during social female-directed singing in Area X. However, we found significantly higher levels of *OTR+* cells that were *EGR1+* during non-social undirected singing compared to social female-directed singing. These data suggest that a higher proportion of Area X neurons expressing the oxytocin receptor are active during non-social undirected singing compared to social female-directed singing. In conclusion, social context information that underlies changes in singing behavior could involve direct oxytocinergic input to Area X, as well as reciprocal connections between the PVN and the social behavior network.

Disclosures: K. Anderson: None. **L. Colon:** None. **V. Doolittle:** None. **O. Whitney:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

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Program #/Poster #: 645.14

Topic: F.01. Neuroethology

Title: Sex differences in responses to song and songlike stimuli in zebra finches

Authors: *A. SAVOY, D. MARGOLIASH;
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Abstract: Differences in neuroanatomy underlie differences in signal processing, perception, and behavior. In songbirds, a long-standing assertion has been that for species in which females

do not sing, the nuclei of the "song system" are vestigial or diminished relative to their male counterparts, though recent evidence shows robust connectivity between these areas in females. One possibility is that this circuitry is involved in female auditory processing and evaluation of song and other vocalizations. This could be highly relevant to one crucial consequence of song perception: female mate choice. We hypothesized that adult female zebra finches would be more behaviorally responsive to novel conspecific song than adult males. We also explored whether artificial songlike rhythms would induce activation or suppression depending on spectral structure and the listener's sex. 30 females and 30 males were individually exposed to multiple identical playbacks of a novel song, as well as four songlike stimuli differing only in spectral structure. We then analyzed differences in amount of vocalization and movement in response to each stimulus type. Our results indicate that females and males differ in their behavioral responses to song and songlike stimuli. Females are more likely to increase their amount of vocalization and movement in response to song, while males are more likely to abruptly cease vocalizing and moving, often remaining suppressed for an extended period. While both sexes show similar decreases in vocalization and movement in response to artificial stimuli, females are more responsive to harmonic stack rhythms. These data collectively suggest that females and males process auditory information differently. We are currently working towards assessing electrophysiological responses in song system and auditory nuclei in response to artificial stimuli and songs of different structure and social significance, probing the neural correlates of these sexually dimorphic behavioral outputs. Sex differences in the way the female brain processes song information may inform mechanisms underlying the effectiveness of courtship song.

Disclosures: **A. Savoy:** None. **D. Margoliash:** None.

Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.15

Topic: F.01. Neuroethology

Support: JSPS Grant 21J20506

Title: Neural bases for song preference behavior to father's song in female zebra finches

Authors: *Y.-C. LIU, Y. MOROHASHI, Y. YAZAKI-SUGIYAMA;
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Abstract: Zebra finches are non-duetting songbirds, in which males sing individually unique song to attract females for courtship. Females recognize individual males and choose mates by discriminating their songs. Females memorize experienced songs and show affiliative behaviors toward familiar songs (usually their father’s song (FS)) over novel songs (Clayton, 1988; Riebel, 2000). More numbers of neurons are activated by FS presentation than novel songs in two distinct telencephalic auditory areas, caudomedial nidopallium (NCM) and caudomedial

mesopallium (CMM), (Terpstra et al., 2006). However, whether these FS-responding neurons are involved in familiar song preference behaviors has yet to be clarified. Here, we investigated if the NCM and/or CMM are neural bases for FS memories and involved in familiar song preference behavior. Females showed significantly reduced preference strength to FS, tested by using perch-hop assay, two weeks after the CMM lesions (N=3) by ibotenic acid injection, while they showed consistent preference strength after the NCM (N=4) or sham lesions (N=6). To further investigate whether FS-responding neurons in the NCM or CMM were involved for familiar song preference behavior, we expressed caspase and diphtheria toxin A that induced cell death in neurons which were activated during doxycycline administration by using the adeno-associated virus (AAV) vectors (AAV-cFos-TetON-CaCasp3 / AAV-cFos-TetON-dtA). Females showed significantly reduced preference strength to FS two weeks after the induction of cell death in the NCM or CMM neurons which were activated by FS playbacks (NCM: N=5; CMM: N=5). Females in which cell death was induced in the FS-responding CMM neurons recovered song preference behavior to FS a month after the induction of cell death, while females induced to cell death in the FS-responding NCM neurons showed similar recovery two months after the induction of cell death. Taken together, those suggest that both the NCM and CMM have neuronal bases for FS memories and those neurons are involved for familiar song preference behavior in female zebra finches.

Disclosures: Y. Liu: None. **Y. Morohashi:** None. **Y. Yazaki-Sugiyama:** None.

Poster

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Program #/Poster #: 645.16

Topic: F.01. Neuroethology

Support: NIH R01NS082179

Title: Topographic, network-level organization of response profiles in the songbird auditory forebrain.

Authors: *F. A. CINI, L. REMAGE-HEALEY;
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Abstract: Auditory learning is a key component of vocal learning and communication. Neurons involved in auditory learning are typically examined as single encoders, but there is increasing evidence that the coincident activity of groups of neurons, or ‘ensembles', is important for the processing and transmission of cortical information. In songbirds, a forebrain region analogous to mammalian secondary auditory cortex, the caudomedial nidopallium (NCM), is crucial for representing and responding to auditory stimuli. In the awake state, NCM neurons adapt to repeated presentation of song stimuli, considered a form of auditory working memory. Furthermore, there is evidence that different subregions of NCM have different molecular

profiles, such as the release of estrogens and catecholamines, as well as differential expression of immediate early genes in response to specific song features. Therefore, we hypothesize that: 1) stimulus adaptation occurs because NCM neurons form ensembles that encode song and sparsify over time; and (2) subregions of NCM work together to collectively represent song features. We examine how NCM subregions differently process song and its spectrotemporal components, and how they work together to form neuronal ensembles. We used 64 channel probes to record single-unit activity systematically across the NCM of awake zebra finches (Taeniopygia guttata). There were two types of stimulus presentation: first, we presented conspecific songs repeatedly in order to induce adaptation, and second, we presented spectrotemporal modulation stimuli designed to isolate receptive-field components of zebra finch songs ('ripples'). Our preliminary data show that units in dorsal NCM adapt more strongly to song than those in ventral NCM. Also, ventral NCM neurons respond more strongly to spectrotemporal modulation ripples, while units in dorsal NCM are more selective for ripple stimuli. In addition, a combination of principal and independent component analyses indicate that multiple ensembles are concurrently activated by song, and single ensembles are activated by different songs. Altogether, these results suggest that NCM has a topographic organization along the dorsoventral axis, consistent with differential functional roles of these subregions in sensory processing. Furthermore, song representations are carried by multiple ensembles in parallel, suggesting communication between sub-regions to fully represent the soundscape. Support from NIH R01NS082179.

Disclosures: F.A. Cini: None. **L. Remage-Healey:** None.

Poster

645. Vocal/Social Communication – Avian I

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Program #/Poster #: 645.17

Topic: F.01. Neuroethology

Support: Kakenhi Grant 22K15233

Title: Investigating Parallel Song Memory Connections in the Zebra Finch Higher Auditory Cortex

Authors: *S. MORSON, Y. MOROHASHI, Y. YAZAKI-SUGIYAMA;
Okinawa Inst. of Sci. and Technol. Grad. Univ., Onna, Japan

Abstract: Like human infants learn to speak through interaction with, and mimicking of, their parent's speech, male juvenile zebra finches learn to sing by listening to and memorizing, and then mimicking their tutor's song by matching their own vocalizations to the previously formed tutor song memory. In the zebra finch, the caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM) comprise a region analogous to the mammalian higher auditory cortex. The NCM has been previously shown as a site of tutor song, while the neighbouring CMM has been also reported to correlate with tutor song memories and directly project to the song motor area,

HVC. While it has been hypothesized that the NCM and CMM may act as parallel auditory memory locations for tutor song, the connections between these two regions and how the locations of tutor song memory may change through the sensorimotor period as birds develop their own crystalized song has yet to be understood. Here, by using the cFos TetON system newly optimized for zebra finches, we labelled the neurons responding to tutor song with EGFP in the NCM and mRFP in the CMM (using AAV2/9 cFos-TetON-EGFP and AAV2/9 cFos-TetON-mRFP) in adult male zebra finches. Our anatomical analysis identified neurons activated by tutor song in both the NCM and CMM. We also performed tracing analysis of tutor song responsive neurons in the NCM which showed projections to the CMM region and those in the CMM reciprocally projected to the NCM. We extended the experiments to identify neuronal representation of bird's own song in the NCM and CMM, as when birds begin to sing their own song they must form memories of them alongside those of tutor song . We combined cFos-TetON/TetOFF to separately label the neurons responding to tutor song with EGFP and the ones responding to the bird's own song with mRFP (using AAV2/9 cFos-TetON-EGFP and AAV2/9 cFos-TetOFF-mRFP) in both the NCM and CMM of adult male zebra finches. We found the distinct neuronal populations were activated by either tutor song, bird's own song, or both, in both the NCM and CMM. These populations did not differ in their proportions between the adult NCM and CMM (n=3 birds, $p>0.05$) implying that they were represented equally in both of these regions in adult male birds. Together these suggest that both the NCM and CMM involve distinct neuronal substrates of song memories and the ones for tutor memory are interacting with each other across these neighbouring regions.

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Poster

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Topic: F.01. Neuroethology

Support: 2R01NS094667-06

Title: Primary auditory cortical signals change across social states in singing birds

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Abstract: Sensory feedback is important for motor control and learning. Songbirds learn to sing through a process of trial and error that requires auditory feedback. When zebra finches practice their songs alone, performance errors are routed from auditory cortical areas to ventral tegmental area (VTA) dopamine (DA) neurons, which in turn project to the song production system to control plasticity. Recently, we discovered that when male birds transition to female-directed song, DA error signals are gated off and instead become strongly driven by female calls. This

courtship context-dependent gating of DA processing could reflect a local process in VTA or, alternatively, could reflect a more brainwide change in auditory processing. To distinguish these possibilities, we recorded from Field L, a primary auditory cortical area, while birds sang alone and then to females as we controlled perceived performance errors with distorted auditory feedback (DAF). We discovered that many Field L neurons changed their song-locked firing at the transition to courtship singing, even though the auditory feedback was highly similar. Surprisingly, though courtship singing uniformly decreases the magnitude of DA error signals, in Field L, the courtship singing state can increase or decrease error responsiveness. Notably, a large fraction of neurons were highly sensitive to female calls during the male song, as previously observed in downstream DA neurons. These findings show, for the first time in a primary sensory cortical area, that social context can affect the processing of sensory feedback from self-produced actions.

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Poster

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Program #/Poster #: 645.19

Topic: F.01. Neuroethology

Support: DFG project 327654276 – SFB 1315

Title: Targeted manipulation of interneuron activity within HVC alters adult zebra finch song

Authors: *F. HEIM[1,2], E. MENDOZA[3], A. KOPARKAR[2,4,5], C. SCHARFF[3], D. VALLENTIN[1,2];
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Abstract: Male zebra finches learn their song as juveniles from an adult tutor during a critical period. This learning process is dynamic but leads to a static maintenance period in adulthood during which the song remains unchanged. The premotor nucleus HVC (proper name) is a key brain region for vocal learning and production in songbirds as it receives auditory information and contributes to motor learning and output. HVC is comprised of four different cell types including premotor neurons and GABAergic inhibitory interneurons which are involved in vocal production. GABAergic interneurons selectively inhibit premotor neurons while birds listen to learnt song elements suggesting that inhibition within HVC serves a potential mechanism to ensure song maintenance in adult zebra finches. To directly test whether HVC interneurons are crucial for song stability in adulthood we administered a GABA-A antagonist on HVC during playbacks of birds own song (BOS) with an altered syllable sequence while simultaneously

monitoring the birds' song production. Lifting the impact of inhibition in HVC resulted in the occurrence of novel elements as well as sequence changes during song production throughout the experiment. These changes occurred temporarily and reverted back to normal after the pharmacological agent's washout period. In order to specifically manipulate interneuron activity during the listening phase, we designed a viral strategy to optogenetically target GABAergic interneurons in a temporally precise manner in freely moving birds. We then exposed the birds to unfamiliar song and simultaneously suppressed interneuron activity while an animal was listening to playbacks but not during song production. This manipulation resulted in the addition of novel elements towards the end of the birds' otherwise unchanged song. Birds receiving the same playback exposure and optogenetic treatment but injected with a control virus did not display changes in their singing behaviour. Our results imply that the inhibitory network in HVC plays an important role for the performance stability of learnt song production in adult zebra finches. Moreover, precise and cell-type specific manipulations of the inhibitory network in HVC have the potential to re-open the song learning phase, a window of heightened brain plasticity, and we thereby, might be able to teach novel and unfamiliar song elements to adult zebra finches well after their critical period for song learning has ended.

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Poster

645. Vocal/Social Communication – Avian I

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Title: Tracing the development of learned song preferences in the female zebra finch brain with functional Magnetic Resonance Imaging

Authors: P. ARYA[1], A. CHEUNG[1], N. H. KOLODNY[2], ***S. M. H. GOBES**[1];
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Abstract: In sexually dimorphic zebra finches (*Taeniopygia guttata*) only males learn to sing their father's song while females learn to recognize the songs of their father or mate but cannot sing themselves. Memory of learned songs is behaviorally expressed in females by preferring familiar songs over unfamiliar ones. Auditory association regions such as the caudomedial mesopallium (CMM) have been shown to be key nodes in a network that supports preferences for learned songs in adult females. However, much less is known about how song preferences

develop during the sensitive period of learning in juvenile female zebra finches. In this study, we used blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) to trace development of a memory-based preference for the father's song in female zebra finches. fMR imaging was performed in juvenile (58 dph) and young adult (91 dph) females, and a phonotaxis test at the end of development was performed to determine the birds' behavioral preferences for learned over unfamiliar song. We identified father-song selective responses in the auditory thalamus (dorsolateral nucleus of the medial thalamus, DLM; part of the anterior forebrain pathway, AFP) in juvenile female zebra finches. In adult female zebra finches, only in birds that showed a preference for learned song over novel conspecific song, neural selectivity for the father's song was localized in both DLM and CMM. These data reveal a role of the auditory thalamus in sensory learning in female zebra finches, and that neural responses in CMM are shaped during development to support behavioral preferences for learned songs.

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Poster

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Topic: F.01. Neuroethology

Support: SFN 31003A_127024
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Title: The premotor population code of birdsong

Authors: *C. LORENZ[1,2], E. M. ARNEODO[3], K. LEE[1], S. KOLLMORGEN[1,4], N. GIRET[2], R. H. R. HAHNLOSER[1];
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Abstract: An important observation from large-scale neural recordings is that population activity resides in a subspace of the overall spanned neural space. For instance, expanding the number of recorded neurons and the stimulus dimensionality, previous research showed that visual responses in mouse visual cortex are of dimensionality constrained by a factor that putatively ensures a smooth underlying manifold (Stringer et al., 2019). Inspired by this study, we asked whether similar constraints exist in motor areas associated with the production of birdsong, a complex learned skill. The method used in (Stringer et al., 2019) of estimating trial-to-trial response variability from repeated presentation of a stimulus cannot be applied in our case, because birds never sing a particular song variant more than once, i.e., there is no trial-to-trial variability purely on the neural level without also entailing behavioral variability. We therefore set out to test for continuity of the premotor neural manifold using a different approach.

We designed a lightweight and reusable implant using Neuropixel probes (Jun et al., 2017) to perform large-scale neural recordings (up to 384 channels) in freely moving adult male zebra finches (*Taeniopygia guttata*). We targeted both the cortical premotor region LMAN and the basal-ganglia-like striatal Area X, which account for a large fraction of observed trial-by-trial motor variability crucial for song learning and adult vocal plasticity (Ölveczky et al. 2005, Andalman and Fee, 2009). We recorded simultaneously neural activity in both areas while the birds were singing several hundreds of song renditions and explored the relationship between the neural population activity and vocal output. In a preliminary analysis on LMAN spiking activity, we applied a non-parametric, neighborhood-based method (Kollmorgen et al., 2019) and found that similar, i.e., neighboring renditions in neural space are closer in song space than randomly selected renditions. This topographic correspondence suggests a continuous mapping between the neural premotor activity and the produced acoustic output. In other words, small changes in neural space consistently relate to small changes in vocal space, indicating a similar embedding scheme as previously found in the sensory domain. The requirement of continuity of the neural manifold will hopefully help us to understand the underlying coding scheme that birds use for vocal learning and control.

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Poster

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Topic: F.01. Neuroethology

Title: Female vocal feedback improves song learning and alters premotor activity in juvenile zebra finches

Authors: *L. BISTERE[1,2], D. VALLENTIN[1,2];
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Abstract: Learned motor behaviors are often shaped by social influences. For instance, human infants learn to speak through the process of observational learning, imitating and integrating parental feedback. Young zebra finches learn their song from their fathers in a similar manner. Since female zebra finches do not sing, their contribution to song learning has been largely neglected. To investigate the role of female zebra finches on juvenile song learning, we used an operant conditioning paradigm to train young male zebra finches to imitate a song playback. The juvenile birds were housed in two different social contexts: in isolation or together with a female bird. Both groups were tutored with the same tutor song that birds elicited themselves via key pecks. We tracked the song learning trajectories of juveniles in both groups and found, that female presence during the song learning phase increased the similarity of learnt song to the tutor

song. Furthermore, the syllable rate of the tutor song was best matched by the group housed with a female. Although female birds do not sing, they produce calls which can be the source of auditory feedback to guide juveniles' song learning. To explore whether female vocalizations have the potential to elicit changes in the premotor circuitry necessary for song learning and production, we performed intracellular recordings in awake, listening juvenile birds in the premotor area HVC (proper name). A subset of HVC premotor neurons modulated their spiking activity in response to female calls. Additionally, spiking precision was increased when birds own song playback was accompanied with a female call. In adults, it has been shown that female call presentation during birds own song does not change the spiking activity of HVC premotor neurons. To investigate the developmental change responsible for this switch, we performed intracellular recordings in adult birds under anesthesia, a state of decreased inhibition. In this case we found that the time-locked responses to the female calls during birds own song playback reemerged even in adult birds. Together these data suggest, that the presence of a female during song learning leads to increased tutor song matching performance and that female vocalizations have the potential to elicit changes in premotor circuitry leading to song improvements in learning juvenile birds.

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Poster

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Topic: F.01. Neuroethology

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Title: Songbirds need daily song to maintain peak vocal performance

Authors: *I. ADAM[1], N. WOOD[3], P. STÅL[4], K. RIEBEL[5], M. PREVIS[3], C. P. ELEMANS[2]; [2]Biol., [1]Univ. of Southern Denmark, Odense M, Denmark; [3]The Univ. of Vermont, Burlington, VT; [4]Umeå Univ., Umeå, Sweden; [5]Leiden Univ., Leiden, Netherlands

Abstract: Juvenile songbirds learn to imitate a high fidelity copy of their tutor's song. Over this 60-day period of sensorimotor learning, next to the brain, their peripheral sound producing system also undergoes significant functional changes, e.g., the mass of male's vocal muscle increases and they attain the fastest contractile kinetics of any vertebrate muscle. Whether these changes are caused by a developmental program, hormonal influences or vocal muscle training remains unknown. Here we test the hypothesis that superfast muscle speed is achieved due to extensive singing training. Both muscle performance and anatomy of vocal muscles stayed at juvenile levels after muscle use was fully prevented by denervation, which supports the hypothesis that changes are induced by muscle use. To test whether adults need to train for

maintenance of superfast muscle kinetics, we prevented use by denervation and singing prevention. In both paradigms, the speed of vocal muscles and their force production decreased within days. Proteomics analysis showed the mechanism driving these changes is downregulation of proteins involved in force production, speed and ECC calcium handling. Furthermore, we show that song preventions drives changes in the male song. Female preference tests revealed that females first can detect these changes, but more importantly females preferred normal song over song by the same individual after not singing for a week. Taken together, our data show that vocal muscle performance and maintenance is driven by use and that juvenile and adult male birds need to sing daily to gain and upkeep their extreme vocal performance. Furthermore, our data provides a novel mechanistic explanation for song being an honest signal for male quality.

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Poster

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Program #/Poster #: 645.24

Topic: F.01. Neuroethology

Title: Development of behavioral experimental system for courtship song discrimination in songbirds

Authors: *M. IWASAKI, S. NOGUCHI, M. INDA, K. HOTTA, K. OKA;
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Abstract: The males of zebra finch (*Taeniopygia guttata*), a species of songbird, sing a courtship song to the females. The courtship song differs among individuals, and females identify the song and make a pair with the male singing their preferred song. The song discrimination behavior of females has been conventionally evaluated in operant conditioning behavioral experiments. These experiments have revealed that songbirds can discriminate among presented song stimuli with a high rate of correct answers (Nagel *et al.*, 2010; Narula & Hahnloer, 2021; Paul *et al.*, 2021). On the other hand, it has been reported that songbirds make the wrong choice from about first 20% of the time sequence of the song (Nagel *et al.*, 2010). From this fact, it is possible to make a *hypothesis;* songbirds listen to only the first part of a song and lead to misidentification of the song. However, this misidentification generally has not been well studied. In this study, we developed a behavioral experimental system to investigate the *hypothesis* for bird song identification. We have controlled the total experimental system for operant conditioning behavioral using Arduino. In this experimental system, birds are asked to identify two courtship songs. If the bird correctly identifies them, an automatic feeder is opened and the bird is rewarded with food. The birds were trained to perform this identification task in four stages; song mode, food mode, sequence mode, and discrimination mode. In song mode, the birds

learned that song was played back when they hopped on the song perch. In food mode, the birds had to hop on one of the perches to access the food. In sequence mode, the bird had to move to one of the response perches within 7 seconds of hopping on the song perch. Finally, in discrimination mode, classification trials were started. Despite these four stage learning, no birds learned the identification task. Many birds continued to perch on the perch to play back the song and did not perform the task to obtain the food even after starting the trial. We suppose these results indicated that food was not a sufficient reward for the birds. Therefore, we devised an experimental system in which song was used as a reward. The type of song played depends on the perch on which the bird perched, and the preference for the song was determined by the number of times the bird perched on the corresponding perch. and the bird's preference was determined by the number of times it perched on which perch. This experiment could be conducted with three birds, and we were able to establish a new experimental system in which the birds acted on their own to listen to the song.

Disclosures: **M. Iwasaki:** None. **S. Noguchi:** None. **M. Inda:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

645. Vocal/Social Communication – Avian I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 645.25

Topic: F.01. Neuroethology

Support: Project grant from the Swiss national Science Foundation 31003A_182638, 'The roles of vocal communication in pair formation and cultural learning in songbirds' Swiss national Science Foundation NCCR Evolving Language, Agreement #51NF40_180888

Title: Radio Receiver and Audiovisual Data Acquisition System for Longitudinal Recordings of Groups of Songbirds.

Authors: *L. RÜTTIMANN[1], J. RYCHEN[1], T. TOMKA[1,2], H. HÖRSTER[2], M. D. ROCHA[1], R. H. R. HAHNLOSER[1,2];
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Abstract: The study of animal behavior provides a wealth of information on important issues such as animal welfare and it can contribute to our understanding of the workings of the brain. Longitudinal observations of animal groups have benefited from advances in sensing technology and data science methods. Multi-modal recording systems allow resolving individual animals and their complex interactive behaviors such as their vocal gestures. For such systems to be fault tolerant and maximally useful, the various data streams need to be well synchronized and exhibit some level of redundancy.

To enable longitudinal recordings of songbirds in groups of up to eight birds, we have designed a behavioral recording setup driven by a single clock, in which we record video with cameras from multiple viewpoints, sounds with stationary microphones, and body vibrations with custom ultra-low power animal-borne wireless transmitters. These devices specifically sense vocalizations of an individual and transmit the vibratory signals as frequency-modulated (FM) radio waves. To receive the highly variable radio signals, we designed a custom software-defined radio recorder that uses a novel multi-antenna demodulation algorithm, which reduces the radio signal loss rate by a factor of 20 to only 0.12% of the recording time and increases the signal-to-noise ratio of the radio signal by 6 dB on average. To verify the reliability and source separation enabled by the vibratory sensors, we have segmented vocalizations produced by pair-housed birds. We find that neither the vibration sensor nor a single stationary microphone is sufficient by itself to signal the complete vocal output of an individual. Either sensor misses about 3.5% of vocalizations compared to our reconstruction of vocal output combining all recorded channels. As we expect the miss rate from individual sensors to increase with the number of interacting birds in the setup, the benefits of our recording system likely increase along with the size of the social group.

Disclosures: L. Rüttimann: None. **J. Rychen:** None. **T. Tomka:** None. **H. Hörster:** None. **M.D. Rocha:** None. **R.H.R. Hahnloser:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.01

Title: WITHDRAWN

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.02

Topic: F.03. Stress and the Brain

Support: MH113128
MH093650
AA025038
AA026051

Title: Role of pituitary adenylate cyclase activating peptide (PACAP) in the effect of chronic social defeat stress in mice

Authors: *L. LEPEAK, J. RAUH, J. MARQUEZ, P. COTTONE, V. SABINO;
Boston Univ., Boston, MA

Abstract: Role of pituitary adenylate cyclase activating peptide (PACAP) in the effect of chronic social defeat stress in mice
AUTHORS: L. LEPEAK, J. Rauh, J. Marquez, P. Cottone, V. Sabino; Boston Univ. School of Medicine, Boston Massachusetts
DISCLOSURES: L. Lepeak: None, **J. Rauh**: None, **J. Marquez**: None, **P. Cottone**: None**, V. Sabino**: None.
Inability to cope with stressful events can precipitate or exacerbate several psychopathologies, including anxiety disorders and post-traumatic stress disorder (PTSD). The neurobiological mechanisms underlying the response to chronic stress remains, however, poorly understood. Stress neuropeptides systems in the extended amygdala, which mediate the behavioral response to stress, may become hyperactive following chronic stress and drive the emergence of persistent negative outcomes. In this study, we focused on the neuropeptides pituitary adenylate cyclase activating peptide (PACAP) and calcitonin gene-relating peptide (CGRP), both of which play a key role in stress regulation. We used the behavioral paradigm chronic social defeat stress (CSDS) as a relevant model of chronic psychosocial stress in mice and subsequently examined changes in the levels of these neuropeptides in the extended amygdala and in the upstream projecting area parabrachial nucleus (PBn). We found that 10 days of CSDS cause a significant increase in PACAP immunoreactivity within the CeA, but not the BNST, of male mice, compared to control, unstressed mice. We also found increased levels of PACAP in the lateral PBn of CSDS mice, compared to controls. Notably, 10 days of CSDS caused a significant reduction in CGRP levels in the lateral PBn, a peptide that has been proposed to be co-expressed with PACAP in this region. In addition, we are currently beginning investigating the mechanism of action of PACAP in the CeA, with a focus on protein kinase C δ, the marker of a CeA neuronal population that has been shown to mediate aversion, anxiogenesis and nociception, and on corticotropin-releasing factor (CRF). These findings support the notion that the activation of the PACAP system in the CeA originating in the PBn may mediate the behavioral outcomes of chronic psychosocial stress, while. the role of CGRP appears to be dissociated from that of PACAP and perhaps more complex. This research may lead to the development of new therapeutic targets for the treatment of anxiety disorders and PTSD.

Disclosures: L. Lepeak: None. **J. Rauh:** None. **J. Marquez:** None. **P. Cottone:** None. **V. Sabino:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.03

Topic: F.03. Stress and the Brain

Support: CZ.02.2.69/0.0/0.0/19_073/0016935

Title: Nicotinic activation of Neuropeptide Y-expressing neurons in feeding and stress

Authors: *A. ABBONDANZA[1,4], M. DRAPSIN[2], M. RUSKOVA[3], S. DUMAS[5], V. BERNARD[4], A. SUMOVA[2], H. JANICKOVA[1];
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Abstract: Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the brain, where it can be secreted, acting as effector both at pre- and post-synaptic level or it can serve as a marker to identify specific neuronal population, such as, GABAergic interneurons. The diversification of NPY's functions follows its expression. For instance, NPY might modulate fear memory or anxiety via amygdalar circuits or regulate energy homeostasis and stress response via hypothalamic nuclei, including the arcuate nucleus (ARC). On the other hand, within the ARC, the orexogenic NPY/Agouti-related peptide (NPY/AgRP) neurons are responsive to variation of the metabolic state, hormones, GABAergic and glutamatergic transmission, and nicotine. Interestingly, nicotine consumption is classically associated with a reduction of food intake and an increase of the hypothalamo-pituitary-adrenal stress response, whereas NPY release to the hypothalamic paraventricular nucleus (PVN) stimulates feeding behavior. Given a dysmorphic diversity in feeding, we decided, at first, to investigate the link between NPY and beta2-containing nicotinic acetylcholine receptors (b2-nAChRs) expression both in males and in females. We used double fluorescent in situ hybridization for NPY and b2-nAChRs to localize their co-expression patterns at different embryonic days and in adulthood, in both sexes. Then, to understand if cholinergic activation of NPY-expressing neurons can modulate NPY release, we crossed beta2-flox/flox mice with NPY-Cre-IRES to obtain the deletion of b2-nAChRs in all the NPY-expressing cells. We performed feeding behavior analysis, coupled with evaluation of metabolic parameters like glucose tolerance and weight gain. In addition, we used the restrain method for stress induction and we measured the susceptibility to stress in our mice by evaluating the behavioral outcomes in elevated plus maze, open field, social interaction tasks and forced swimming test. In females, the deletion of b2-nAChRs increases the susceptibility to daily stressors and impairs the preference for social interaction, while in males it influences metabolism and body weight. The phenotype observed suggests a more complex interplay between the cholinergic system and NPY release, with the possible recruitment of other types of nicotinic receptors and compensatory mechanisms, that need further investigation.
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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.04

Topic: F.03. Stress and the Brain

Support: NIH Grant R01MH123544

Title: Chronic stress and its effects on behavior, RNA expression of the bed nucleus of the stria terminalis, and the M-current of NPY neurons.

Authors: *T. DEGROAT[1], K. WIERSIELIS[1], K. DENNEY[3], J. TOLLKUHN[3], B. A. SAMUELS[2], T. A. ROEPKE[1];
[1]Animal Sci., Rutgers Univ., New Brunswick, NJ; [2]Psychology, Rutgers Univ., Piscataway, NJ; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: In humans, chronic stress leads to the development of mood disorders such as major depressive disorder and post-traumatic stress disorder. It has also been shown that women are more susceptible to the development of these disorders, suggesting a sex-related difference in how we process stress. The bed nucleus of the stria terminalis (BNST) is a brain region that is essential for the central stress response. It is considered a part of the extended amygdala and serves as a relay between the amygdala and the prefrontal cortex. This region is also sexually dimorphic in its expression of aromatase and estrogen receptors. Therefore, the BNST may play a major role in the sex difference observed. Furthermore, Neuropeptide Y (NPY) is highly expressed in the BNST and plays a role in stress. To study the effects of chronic stress on the BNST, we used both wild-type male and female mice and male and female mice tagged with GFP in the NPY promotor. These mice experienced six weeks of a chronic variable mild stress (CVMS) paradigm prior to behavior, tissue collection for RNA sequencing, and whole-cell patch clamp electrophysiology. The behavior tests we conducted were the open field test (OFT), elevated plus maze (EPM), light dark box (LDB), and novelty suppressed feeding (NSF). We hypothesized that the CVMS paradigm would results in alterations in behavior of both male and female mice, sex-dependent differences in the transcriptome, and that NPY neurons would be affected by chronic stress, leading to a decreased M-current. We also hypothesized that stressed females would be more sensitive than males. Our results show that stress did cause sex-dependent differences in behavior depending on the parameters of the test. In the OFT, CVMS mice spent less time in the center and more time in the corners. In the EPM, CVMS exposed mice spent more time in the closed arms than controls and females had more open arm crossings than males. In the LDB no significant effects were observed other than male mice had significantly more stretch attend postures than females. In the NSF, no significant effects were observed. CVMS exposure did not affect the M-current in the NPY neurons, nor was there a sex-related difference. The max peak of the M-current in control males was 30.4 pA, in stress males max peak was 34.0 pA, in control females max peak was 23.4 pA, and stress females max peak was 26.8 pA. RNA Sequencing data is currently being analyzed. This suggests that CVMS is a potent effector of behavior in mice, but that BNST NPY neurons may not be an important regulator of chronic stress or a mediator of the sex differences in the response to chronic stress.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.05

Topic: F.03. Stress and the Brain

Support: ORWH-U54-MH118919

Title: The effects of an acute stressor on the regulation of gene expression in the hypothalamic paraventricular nucleus

Authors: *M. ROUEINFAR[1], R. HANDA[1], S. TOBET[1], K. A. FRAHM[2];
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Abstract: Stress has persistent effects on the activation of the hypothalamic-pituitary-adrenal (HPA) axis, a major neuroendocrine axis regulating mammalian homeostasis. Neurons in the hypothalamic paraventricular nucleus (PVN) play critical roles in the stress response by regulating pituitary release of adrenocorticotrophic hormone. Stressors often impact gene expression across the HPA axis in a sex-selective pattern, but mechanisms maybe hormonal or genetic or both. To assess stress effects on gene expression and identify genes that are essential in regulation of homeostasis, adult (3-6 mo) wildtype C57BL/6J female and male mice were divided into control and stress groups. PVNs were dissected and analyzed via RNA sequencing (RNAseq). Control mice were euthanized directly out of their home cage and the stress groups were euthanized at 90 min recovery (n = 3-4 groups) after 2h of multi-modal stress (restraint, loud music, light and shaker). Total RNA from PVN was analyzed using RNAseq and data showed that sex-specific genes such as *Kdm5d, Uty*, and *Ddx3y* were differentially expressed between the two sexes. We also identified stress induced and GR-target genes that are either regulated equivalently in male and female (e.g., *Fkbp5* and *Sult1a1*) or differentially responsive in in males (e.g., *Zbtb16* and *Mertk*) or females (e.g., *Plin4* and *Gpd1*). It has previously been reported that *Zbtb16* is induced under various stress conditions via GR signaling in PVN (Cheng et al., *Front. Neurosci. 2020; 14:592947)*. Loss of *Zbtb16* has been shown to increase the expression in inflammatory cytokines (Sadler et al., 2014: PNAS; 112:1535). Further RNA-Seq analysis and gene ontology revealed that multiple members of *ADAM* family were down-regulated and that anti-inflammatory cytokines such as *TNFaip8l3* and *TNFaip6* are up-regulated in our RNAseq PVN data, consistent with an altered immune response. Ongoing studies are examining the molecular circuit involved in regulation of GR-induced stress response and potential bases for sex specific differences in those genes. Given the contribution of maternal immune activation to prenatal programming of adult susceptivity to psychiatric disorders, these immune pathways may play key roles in stress responses.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.06

Topic: F.03. Stress and the Brain

Support: NIH GM134732

Title: The canonical HPA axis contributes to locomotion during photoadaptation but is not required

Authors: *H. B. LEE[1], V. DANG THI[2], G. E. BOYUM[3], R. MODHURIMA[3], E. M. HALL[4], I. K. GREEN[5], E. M. CERVANTES[1], S. SHAMS[1], K. J. CLARK[1];
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Abstract: The hypothalamic-pituitary-adrenal (HPA) axis and its effector molecules—glucocorticoids— modulate diverse aspects of physiology in vertebrates. While the glucocorticoid receptor (*nr3c1*) is known to be involved in photoadaptation of the retinal cells, the role of HPA axis receptors in the behavioral phenotypes during photoadaptation have not been delineated. Therefore, we investigated locomotor adaptation to various light durations using larval zebrafish that carry a mutated allele in key HPA axis receptors. First, we established baseline locomotion of wildtype (WT) and *nr3c1* mutant larvae in constantly lit and dark conditions (for 12-hrs). WT larval zebrafish showed highest locomotor activity in the middle of the day and low activity during the early and later parts of the day, which was generally higher in the light. The baseline locomotion was depressed in *nr3c1* mutants throughout the day in both environments. Next, groups of larvae mutant in *nr3c1*, *nr3c2* (mineralocorticoid receptor), or *mc2r* (melanocortin receptor type 2; adrenocorticotropic hormone (ACTH) receptor) along with their WT siblings were acclimated in the dark and underwent four cycles of dark-light illumination changes with different durations of illumination: 7.5, 6, 4, or 2 min. The *nr3c1* and *mc2r* mutant fish showed decreased locomotion during the dark phase when the illumination was provided for 4 or 2 min. However, with 4 min or longer illumination, they demonstrated a "catch-up" phenotype with increasing locomotion during the later dark phases of the assay, ultimately reaching the swimming distances indistinguishable from their WT siblings with 7.5-min illumination. Finally, we looked at effects of light intensity. A lower intensity of light failed to elicit any response even from WT fish after 1-min illumination. However, this dim light still evoked a robust response from *nr3c1* mutants and their WT siblings after 7.5-min illumination. The *nr3c2* mutant larvae showed locomotor response similar to their WT siblings regardless of the length of illumination. Thus, activation of the canonical HPA axis (i.e. *nr3c1*, *mc2r*) was necessary to induce a rapid locomotor adaptation following light to dark transition with shorter light exposure (≤4 min), but not needed for locomotor responses after longer exposure to light (> 4 min). Moreover, the locomotor response after transitioning to dark from a short (1 min) exposure was dependent on the intensity of light, whereas the longer exposure was not. These findings suggest that either parallel independent pathway(s) are responsible for the locomotor

response or that the HPA axis facilitates a primary pathway to increase sensitivity to light exposure.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.07

Topic: F.03. Stress and the Brain

Support: University of Colorado Boulder 11004285

Title: Bnst circuitry in stress-induced changes on exploratory behavior

Authors: *A. LY[1], D. MCGOVERN[2], C. FORD[4], D. H. ROOT[3];
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Abstract: Stress can induce long-lasting behavioral changes that result in psychiatric illnesses, such as post-traumatic stress disorder (PTSD). Some of these changes include avoidance of future situations that may be stress-inducing or ignoring one's own basic needs, such as eating. In mice, stress can manifest in similar ways: after a stress-inducing experience, mice will not explore for food or eat in a novel, brightly lit environment in spite of hunger. This phenomenon is known as novelty-suppressed feeding or hyponeophagia. The bed nucleus of the stria terminalis (BNST) is a region of the extended amygdala that regulates behavioral responses to unpredictable or uncertain aversive stimuli. BNST neural activity is also enhanced in response to uncontrollable stress in humans. Using a mouse model of stressor controllability, our preliminary data establishes that both GABA and glutamate activity within the BNST is increased during uncontrollable stress, extending the previously aforementioned finding in humans to specific BNST cells. We have also found that BNST neurons form synapses onto the arcuate nucleus (ARC) and paraventricular hypothalamus (PVN), brain regions that play a central role in feeding and stress regulation, respectively. Using RNAscope *in situ* hybridization, we discovered that the majority of glutamatergic BNST neurons co-express the genetic machinery to vesicularly package both GABA and glutamate. Our preliminary evidence suggests that BNST glutamatergic neurons, defined by VGluT3 expression, functionally release both GABA and glutamate on downstream ARC neurons. Our results may identify novel mechanisms of neurotransmitter co-transmission that may be used to reduce the effects of stress on exploratory behavior.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.08

Topic: F.03. Stress and the Brain

Support: NHMRC CDF 1166123

Title: Investigating orexin involvement in stress-induced binge eating in female mice

Authors: *M. MUTHMAINAH[1,2], M. O'SHEA[1,3], R. ANVERSA[1], P. SUMITHRAN[5], A. GOGOS[1], R. BROWN[1,4];
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Abstract: Stress and negative affect (e.g. sadness, anger, loneliness) are known to trigger overeating, particularly in women. This form of maladaptive eating behaviour, commonly referred to as "emotional eating", is associated with binge eating and higher risk of obesity. The neural mechanisms that underpin this form of dysregulated eating are yet to be elucidated but likely implicate neuronal substrates involved in both homeostatic and hedonic feeding. The orexin (hypocretin) system has been previously implicated in reward, stress and feeding. Thus, we aim to investigate the role of the orexin system in stress-induced binge eating in females. We hypothesised that orexin neurons would be significantly activated as a result of stress-induced binge eating as compared to control and that systemic blockade of orexin 1 receptors will ameliorate this behaviour, thus implicating orexin signalling at orexin 1 receptors in stress-driven maladaptive eating. Mice were subjected to a protocol that employed a mild psychological stressor and intermittent access to a highly palatable food reward to induce binge eating in mice. Mice were administered either the orexin 1 receptor antagonist SB-334867 (15 mg/kg, sc) or vehicle (5% DMSO in saline) on test day. Vehicle-treated mice exposed to a frustrative stressor consumed significantly more of the food reward compared to control mice. The same was not observed for mice that received SB-334867 (15 mg/kg, sc). Further, brain slices from the lateral hypothalamic area of stress binge and control mice were processed for Fos and orexin immunostaining. Early analysis is indicative of a trend toward significant neuronal activation of orexin neurons as a result of stress-induced binge eating. Full quantification of immunohistochemical data is ongoing. Collectively, these data suggest a role for orexin signalling at the orexin 1 receptor in stress-induced binge eating in females

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

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Program #/Poster #: 646.09

Topic: F.03. Stress and the Brain

Support: 2021R1A2C3007164
2022M3E5E8016325
SSTF-BA2201-12

Title: Restraint stress changes the excitability of hypothalamic POMC neuron and food intake

Authors: *G. HA, G.-H. KIM, R. SONG, E. CHEONG;
Yonsei Univ., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Stress activates the hypothalamic-pituitary-adrenal system and induces the release of glucocorticoids, stress hormones, into circulation. Many studies have shown that stress affects feeding behavior. However, the underlying circuitry and molecular mechanisms are not fully understood. Orexigenic (simulating appetite) and anorexigenic (loss of appetite) signals reciprocally modulate feeding behavior. It is suggested that proopiomelanocortin (POMC) and neuropeptide Y (NPY) neurons in the arcuate nucleus (ARC) of the hypothalamus are the first-order neurons that respond to the circulating signals of hunger and satiety. Here, we examined restraint stress model and observed an alteration of food intake. We investigated whether stress affects the properties of POMC and NPY neurons in ARC and found that restraint stress changed the excitatory inputs onto POMC neurons and the action potential threshold, leading to changes in feeding behavior. Additionally, we have further studied to find out the effect of restraint stress in other brain regions related to regulate appetite and satiety.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

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Program #/Poster #: 646.10

Topic: F.03. Stress and the Brain

Support: NIH Grant F99NS120599
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Title: The role of protein kinase C-delta-expressing neurons in the bed nucleus of the stria terminalis in stress-responses and anxiety-like behaviors

Authors: *K. WILLIFORD[1], A. TAYLOR[1], J. BROWN[2], J. MELCHIOR[2], N. PETERSEN[1], M. BEDENBAUGH[1], M. NEGASI[1], E. SALE[1,3], D. WHITEHEAD[1], R. SIMERLY[1], S. PATEL[4], D. WINDER[1];
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Abstract: Chronic stress exposure is implicated in psychiatric disorders such as PTSD, anxiety, depression, and addiction. The bed nucleus of the stria terminals (BNST) is part of the extended amygdala known to mediate many stress responses and anxiety-like behaviors that may contribute to these disorders and the cycle of relapse characteristic of substance use disorders. The BNST contains numerous cell types distinguishable by the expression of distinct neuropeptides and proteins, and evidence suggests that cell-type contributes unique facets to stress responses. BNST cells expressing protein kinase C-delta (BNST(PKCd)) are an abundant and largely distinct population of neurons from other BNST subpopulations, but their function remains under-characterized. In the central nucleus of the amygdala (CeA), a related member of the extended amygdala highly interconnected with the BNST, there exist many cell types that parallel those found in the BNST and which have overlapping functions. CeA(PKCd) cells have been implicated in increasing behaviors including fear learning, pain processing, and compulsive alcohol seeking, and in the BNST, we have found that expression of PKCd is dynamically regulated by stress, suggesting BNST(PKCd) cells may be critical modulators of stress responses and anxiety-like behaviors. In order to investigate the function of BNST(PKCd) neurons, I first used microscopy and fiber photometry to characterize the activation of this population following stress exposure and in real-time during stress, finding that BNST(PKCd) cells show increased activation during instances of active stress coping. I next used *in vivo* optogenetics to determine the impact of activating these cells on anxiety-like behaviors, and found that BNST(PKCd) cell activation is aversive and increases anxiety-like behaviors. Finally, I utilized rabies-mediated tracing and whole-brain imaging to situate these cells in their broader circuit-based context, finding that BNST(PKCd) cells receive afferents from regions involved in affective processing, sensory perception, and consumption behaviors. My ongoing work is investigating a potentially specific role of BNST(PKCd) cells in mediating responses to chronic and/or particularly intense stressors, and the effect of both acute and chronic stress on their afferent control. By elucidating the dynamic, cell-type-specific role of BNST(PKCd) cells, we will provide a more comprehensive understanding of the mechanisms regulating anxiety-like behaviors and other stress-related disorders.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.11

Topic: F.03. Stress and the Brain

Support: NSF Grant 1754513

Title: Social Regulation of the Posterior Tuberal Nucleus in Zebrafish (Danio rerio)

Authors: *F. K. HEAGY[1], K. N. CLEMENTS[1], E. I. BLAIN[1], M. C. SETNESKA[1], F. A. ISSA[2];
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Abstract: Aggression is an important behavioral feature that facilitates the formation of stable dominance relationships. Aggressive animals are recognized as dominants and have priority to resources, while those that display submissive behavior are recognized as subordinates. Despite its social benefits, persistent aggression is stressful psychologically and physiologically. Although the effects of social stress have been investigated, little is known of how it induces morphological plasticity of brain nuclei involved in regulating motor circuits. Here we investigated the effects of socially induced stress on the morphological plasticity of hypothalamic posterior tuberal nucleus (PTN) using adult male zebrafish as a model organism. The PTN is an integration center of multimodal sensory social cues, and its dopaminergic neurons project their axons caudally into the spinal cord to regulate the Mauthner mediated startle escape and swim behaviors. We hypothesized that the PTN is prone to socially induced morphological plasticity that would influence the modulation of motor behaviors in a social status-dependent manner. To test this hypothesis, we measured the sensitivity of the startle response and spontaneous swimming activity in dominant and submissive fish. We found the sensitivity of startle escape was significantly enhanced in submissive animals compared to dominants or communals (control); while swimming was significantly reduced in submissive animals compared to dominants. Histological analysis using *Tg[DAT:eGFP]* transgenic line with targeted eGFP expression in dopaminergic neurons showed a significant reduction in the number of PTN cells in submissive animals compared to dominants and control groups after 14 days of social interactions but no differences between dominants and controls. Digital rendering and volumetric analysis of PTN soma size showed no significant difference among the three social groups. The result suggests that chronic social stress induced neuronal loss that correlates with differences in motor activity. Currently, we are conducting time course experiments to quantify PTN cell number during the first two weeks of interactions to determine whether differences in PTN cell number are a result of social stress rather than being inherent. Additionally, we are staining for cleaved caspase and PSD-95 to assess whether socially induced neuronal loss is accompanied with dendritic atrophy. The results improve our understanding of how social stress induces morphological plasticity of social decision-making networks.

Disclosures: F.K. Heagy: None. **K.N. Clements:** None. **E.I. Blain:** None. **M.C. Setneska:** None. **F.A. Issa:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.12

Topic: F.03. Stress and the Brain

Title: The role of lateral hypothalamus corticotropin releasing factor-expressing neurons in the effects of chronic stress on motivation and feeding behavior

Authors: *A. BAZER, B. A. SAMUELS;
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Abstract: The lateral hypothalamus (LH), which regulates feeding,stress, reward, and motivation behavior, is a heterogeneous structure withdistinct cell populations defined by expression of specific neural markers. Onesuch population are corticotropin releasing factor (CRF) neurons, which areimplicated in chronic stress. However, these CRF neurons are poorlycharacterized and their impact on feeding behavior and motivation is unknown.This study aimed to characterize CRF neurons in the LH by comparingco-expression of VGat and VGlut2 mRNA through in situ hybridization (RNAscope).We also aimed to assess the function of CRF neurons using chemogenetics.CRF-ires-cre mice injected with either Gq-DREADD, Gi-DREADD, or control wererun through three behavioral paradigms (free access feeding, reward preferencefeeding, and effort-related choice) to gauge appetite, desire for reward, andmotivated behavior. CRF neurons in the LH were found to be both glutamatergicand GABAergic. Activation of LH CRF neurons via Gq-DREADD mediation increasedfeeding but did not have an impact on motivation behavior. Further research isnecessary to understand the role of LH CRF neurons in feeding behavior.

Disclosures: A. Bazer: None. **B.A. Samuels:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.13

Topic: F.03. Stress and the Brain

Support: NIH Grant HL135562
UCLA School of Nursing Pilot Funding

Title: Anxiety reduction through engaging interoceptive and breathing rhythm networks: a pilot study of breathing meditation and controlled breathing

Authors: *P. MACEY[1], F. MARTINEZ[2], B. KRAUSE-SORIO[4], D. G. GHAHREMANI[3], P. SIDDARTH[2], H. LAVRETSKY[2];
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Abstract: Breath-based mind-body practices are known to reduce anxiety, but while mind-body treatments are popular, the selection of the best individualized approach is difficult because the mechanisms of action are unclear. One unifying characteristic of breath-based mind-body interventions is interoception, which is the sensing and processing of signals from within the body. Specifically, interoception is engaged by mind-body practices that involve breath awareness. However, other behavioral interventions that are not solely interoceptive can also can reduce anxiety and improve well-being, including slowing the breath based on an external cue, a form of controlled breathing (CB). CB engages some degree of interoception, but also likely influences the brain through a direct breath pacing neural mechanism, since breathing rhythms have been shown to be represented in many brain structures. In a pilot study, we compared CB via paced breathing to breathing awareness (BA) to distinguish the role of interoception (BA) and breathing rhythm (CB) on neural regulation of emotion and anxiety networks. A third group passively watched a nature video (NV), as an exteroceptive and relaxation control. Using fMRI, we studied functional connectivity between brain structures known to regulate anxiety (amygdala), interoception (insula) and breathing rhythms (locus coeruleus; LC). Thirteen participants received resting fMRI scans before and after 8 weeks of near-daily practice of BA (N=7), CB (N=3), and NV (N=3). Additionally, three participants performed BA, CB and NV in the scanner at baseline. We found that during BA and CB, connectivity between the insula (interception) and amygdala (anxiety) was increased versus NV and rest (BA r=0.52, CB r=0.54, NV r=0.39, rest r=0.35). During CB, connectivities between the LC (breathing rhythm) and insula and amygdala were increased relative to other conditions (LC-insula BA r=0.11, CB r=0.35, NV r=0.04, rest r=0.04; LC-amygdala BA r=0.13, CB r=0.35, NV r=0.05, rest r=0.14). After 8 weeks of daily practice, connectivities between the LC and amygdala were increased with CB (r +0.18) but not BA (r +0.01) or NV (r -0.03), and insula-amygdala connectivities were greater in CB (r +0.24) vs BA (r+0.10), and BA vs NV (r -0.11). These pilot data show of the CB, BA and NV interventions, and show that CB and BA may impact the brain differently. More generally, different breath-based mind-body practices may elicit neural effects specific to interoceptive or breathing rhythm networks. Future studies should evaluate whether such neural changes underlie the commonly observed reductions in anxiety with mind-body practices.

Disclosures: P. Macey: None. **F. Martinez:** None. **B. Krause-Sorio:** None. **D.G. Ghahremani:** None. **P. Siddarth:** None. **H. Lavretsky:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.14

Topic: F.03. Stress and the Brain

Support: iTHRIV KL2TR003016/ULTR003015
NIH R01DK132566-01

Title: Threatening stimuli remodel the lateral hypothalamic proenkephalin neurons to promote palatable food overconsumption

Authors: *S. SHIN, I.-J. YOU, Y. BAE, A. BECK;
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Abstract: A threat to safety in a predator-prey interaction is a psychological stressor that induces physiological/hormonal changes, altering overall food intake and appetite preferences. The increased propensity to eat high-calorie "palatable" food particularly appears when the stressful situation ends. However, little is known about specific neural mechanisms that promote high-fat diet (HFD) overconsumption after a threat. Using *in vivo* calcium imaging, we found that proenkephalin (Penk)-expressing-lateral hypothalamic (LH) neurons of mice are highly activated in the presence of cat urine, a predator scent stimuli (PSS). A day after exposure to PSS, the Penk-expressing LH (LH Penk) neurons show more potentiated activity during the first eating bout of HFD, whereas chronic inhibition of the same neurons normalizes the PSS-induced HFD overconsumption. The PSS elevates corticosterone levels, which induces long-term activation of LH Penk neurons. Indeed, pharmacological treatment with corticosterone promotes HFD consumption the next day and elicits a corresponding increase in the activity of LH Penk neurons in response to HFD. Thus, we identify LH Penk neurons as a critical neural substrate comprising threat-induced neuronal adaptation to induce emotional overconsumption of palatable foods following a threat.

Disclosures: S. Shin: None. **I. You:** None. **Y. Bae:** None. **A. Beck:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.15

Topic: F.03. Stress and the Brain

Support: NIH Grant MH059911

Title: Polysynaptic input to glucagon-like peptide 1 neurons in Gcg-Cre rats

Authors: *I. E. GUERRERO, H. ZHENG, L. RINAMAN;
Florida State Univ., Tallahassee, FL

Abstract: The glucagon gene *(Gcg)* encodes preproglucagon, which is cleaved to form glucagon-like peptide 1 (GLP1) by neurons within the caudal nucleus of the solitary tract (cNTS). Central GLP1 neural signaling is implicated in stress-induced arousal, behavioral avoidance, hypophagia, and autonomic responses. We previously reported monosynaptic and

polysynaptic inputs to GLP1 neurons in mice (PMC6891065). The current study aimed to reveal potential differences between rats and mice in polysynaptic inputs to GLP1 neurons. Using our new Sprague Dawley Gcg-Cre rat model, adult rats (9 male, 5 female) received unilateral cNTS injection of a Cre-dependent pseudorabies virus (PRV-introvert) to infect GLP1 starter neurons, followed by retrograde trans-synaptic transport to their first-order and polysynaptic inputs. Rats were perfused 24-123hr later, and brain tissue was processed to reveal PRV-infected neurons. At 24 hr, viral labeling was restricted to the cNTS. Similar to mice, labeling increased at longer survival intervals to include specific regions of the brainstem, hypothalamus, and limbic forebrain. Regions with labeling in rats but not mice included somatosensory and motor cortices. In addition, labeling in the bed nucleus of the stria terminalis and central amygdala was unilateral in mice but bilateral in rats. Overall, our new findings reveal species differences in the organization of central inputs to cNTS GLP1 neurons. Additional studies are necessary to determine the functional impact of these differences.

Disclosures: I.E. Guerrero: None. **H. Zheng:** None. **L. Rinaman:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.16

Topic: F.03. Stress and the Brain

Support: Korea Health Technology R&D Project through the Korea Health Industry Development Institute (No. HI19C0750)

Title: Anti-nociceptive effects of dual neuropeptide antagonist therapy in mouse model of neuropathic and inflammatory pain

Authors: *M. KIM[1], B. KIM[2];
[1]Sch. of Med., Soonchunhyang Univ., Cheonan, Korea, Republic of; [2]Sch. of Med., Wonkwang Univ., Iksan, Korea, Republic of

Abstract: Background: Neurokinin 1 (NK1) and calcitonin gene-related peptide (CGRP) play a vital role in pain pathogenesis, and these proteins' antagonists have attracted attention as promising pharmaceutical candidates. We investigated the anti-nociceptive effect of co-administration of the CGRP antagonist and an NK1 antagonist on pain models compared to conventional single regimens. **Methods**: C57Bl/6J mice underwent sciatic nerve ligation for the neuropathic pain model and were injected 4% formalin into the hind paw for the inflammatory pain model. Each model was divided into four groups: vehicle, NK1 antagonist, CGRP antagonist, and combination treatment group. The NK1 antagonist aprepitant (BIBN4096, 1 mg/kg) or the CGRP antagonist olcegepant (MK-0869, 10 mg/kg) was injected intraperitoneally. Mechanical allodynia, thermal hypersensitivity, and anxiety-related behaviors were assessed using the von Frey, hot plate, and elevated plus-maze (EPM) tests. The flinching and licking

responses were also evaluated after formalin injection. **Results**: Co-administration of aprepitant and olcegepant more significantly alleviated pain behaviors than administration of single agents or vehicle, increasing the mechanical threshold in the von Frey test and improving the response latency in the hot plate test. Anxiety-related behaviors were also markedly improved after dual treatment compared with either naive mice or the neuropathic pain model in the dual treatment group. Flinching frequency and licking response after formalin injection decreased significantly in the dual treatment group. Isobolographic analysis showed a meaningful additive effect between the two compounds. **Conclusions**: A combination pharmacological therapy comprising multiple neuropeptide antagonists could be a more effective therapeutic strategy for alleviating neuropathic or inflammatory pain.

Disclosures: M. Kim: None. **B. Kim:** None.

Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.17

Topic: A.09. Adolescent Development

Support: R01 HD082567
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R01 HD096324
R01 HD043341
P30 DK063491
P30 CA023100

Title: Mutations in SOX2 are a novel hypothalamic cause of Hypogonadotropic Hypogonadism

Authors: *J. CASSIN[1], M. I. STAMOU[2], K. W. KEEFE[2], K. E. SUNG[1], C. C. BOJO[1], K. J. TONSFELDT[1], S. B. SEMINARA[2], R. BALASUBRAMANIAN[1], P. L. MELLON[1]; [1]UCSD, SAN DIEGO, CA; [2]Harvard MGH Ctr. for Reproductive Med. and Harvard Med. Sch., Boston, MA

Abstract: Mutations in *SOX2* are a novel hypothalamic cause of Hypogonadotropic Hypogonadism

Abstract: Isolated Hypogonadotropic Hypogonadism (IHH) is a disorder characterized by low circulating sex steroids and delayed or absent puberty. Mutations in the *SOX2* gene have been previously linked to a syndromic form of IHH with additional ocular and neurodevelopmental phenotypes. The role of *SOX2* variants in non-syndromic forms of IHH remains unclear. To close this gap, we reviewed whole exome sequencing data in a large cohort of IHH (n=1453) patients ascertained by a reproductive phenotype. We identified a total of eight heterozygous *SOX2* rare variants (3 *de novo*) contributing to both syndromic and non-syndromic forms of IHH.

To determine the pathogenicity of the discovered variants, we utilized several *in* vitro methods. First, we confirmed that *SOX*2 is expressed in kisspeptin neurons *in vivo* in the female adult mouse brain and colocalizes with kisspeptin in the anteroventral periventricular nucleus in female mice. We next investigated the effect of SOX2 on kisspeptin expression *in vitro.* We showed that SOX2 binds to the human kisspeptin promoter and represses kisspeptin luciferase expression in two immortalized hypothalamic cell lines, KTaR and KTaV. Of the eight *SOX2* variants identified, four diminished or reversed this repression. Finally, we investigated the molecular mechanism behind the phenotype of each of the four functionally deleterious mutations. Two missense mutations in SOX2 prevent proper localization to the nucleus. Two truncating mutations result in SOX2 fragments that retain their ability to bind DNA and appear to act as dominant negative when titrated in luciferase assays. This study demonstrates that the *SOX2*-related human disease spectrum may include IHH without severe ocular or neurodevelopmental phenotypes. We also show novel mutational mechanisms including dominant-negative effects contributing to *SOX2*-related human disease. This study greatly expands the understanding the mutational spectrum and the underlying mechanisms of the mutations leading to IHH and informs a more complete picture of the complexity of the genetic landscape governing the hypothalamic-pituitary-gonadal axis.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.18

Topic: F.03. Stress and the Brain

Support: NIH R01 DK118292 (YMU)

Title: Interactive effects of palatable feeding and acute stress on endocannabinoid content in stress-regulatory brain regions

Authors: *I. RAINER[1], A. METZGER[2], S. L. BAGLOT[3], C. HUME[4], M. N. HILL[5], Y. M. ULRICH-LAI[2];
[1]Neurosci. Grad. Program, [2]Dept Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH; [3]Neurosci., [5]Hotchkiss Brain Inst., [4]Univ. of Calgary, Calgary, AB, Canada

Abstract: A history of limited palatable “comfort” food intake blunts behavioral and hypothalamic-pituitary-adrenocortical (HPA) axis responses to stress, but the mechanisms underlying this effect are not clear. Brain endocannabinoid (eCB) signaling promotes palatable feeding and blunts stress responses, suggesting that this system is well-poised to mediate the stress relief provided by palatable foods. The present work tests the hypothesis that eCB (N-

arachidonyl ethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG)) content in stress-regulatory brain regions is altered by a prior history of limited sucrose intake (LSI) and/or acute stress exposure in a manner consistent with eCB-mediated stress relief. To test this, adult male Long-Evans rats with *ad libitum* access to water and normal chow underwent the LSI paradigm, in which they were given additional twice-daily access to a small amount (4 ml) of 30% sucrose (vs. water controls) for two weeks. The rats then received an acute restraint stress (vs. no stress controls) immediately prior to the collection of 1) blood for measurement of plasma corticosterone, and 2) the basolateral amygdala (BLA), medial prefrontal cortex (mPFC), hippocampus (HPC) and paraventricular hypothalamic nucleus (PVN) for measurement of AEA and 2-AG content by liquid chromatography-tandem mass spectrometry. The data show that sucrose reduced the plasma corticosterone response to restraint ($p=0.015$). LSI increased AEA (but not 2-AG) content in the BLA and mPFC, after restraint stress (Drink X Stress interaction, $p=0.022$ for BLA, $p=0.04$ for mPFC), consistent with the known stress blunting actions of AEA in these regions. Restraint stress increased 2-AG content in the mPFC, HYP, and PVN, but not the BLA, and LSI did not alter 2-AG content in any brain region. Ongoing work is investigating the extent that these eCB effects replicate in another cohort, extend to another type of palatable food (limited cheese intake), and extend to another type of acute stress (social interaction). Collectively, the results indicate that a history of palatable feeding modulates the rapid changes in brain eCB content that occur following acute stress, supporting eCB signaling as a mechanism for the stress relieving effects of palatable "comfort" foods.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.19

Topic: F.03. Stress and the Brain

Support: NIH Grant R01 MH119814 (YMU/JPH)

Title: The potential role of perineuronal nets of basolateral amygdala parvalbumin interneurons in stress resilience conferred by natural rewards

Authors: *H. NASHAWI[1,2], C. T. FOLTZ[2], M. A. SMAIL[1,2], C. PHARES[2], D. R. BUESING[2], J. P. HERMAN[2], Y. M. ULRICH-LAI[2];
[1]Neurosci. Grad. Program, [2]Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Stress is a major problem in today's society and is a risk factor for many psychiatric and physiological disorders. Engaging in pleasurable activities can improve mood and reduce physiological stress responses, but the neural mechanisms by which positive experiences provide

stress relief are unknown. To study this, we developed a paradigm of rewarding behavior in rats that buffers stress responses; this paradigm provides chronic, twice-daily access to a limited amount (4 mL) of a 30% sucrose solution (limited sucrose intake; LSI). Our prior work implicates the basolateral amygdala (BLA) in the stress-blunting effects of LSI and has shown that LSI increases the expression of plasticity-related genes specifically in inhibitory, parvalbumin-positive (PV) interneurons in the BLA. Since perineuronal nets (PNNs) that surround PV interneurons play a crucial role in regulating their plasticity, we sought to test the hypothesis that LSI confers stress resilience by altering PV PNNs. Adult male Long-Evans rats (n=48) were individually housed and given ad libitum access to regular chow and water. In a 2X2 design, rats were exposed to the LSI paradigm (or provided with an identical bottle of water as a control) for 5 days, following which they were either exposed to a 20-minute restraint stress or moderately handled (as a control) once-daily for 15 days. On day 20, rats were sacrificed and brains were collected for fluorescent immunolabeling of PV interneurons and PNNs. Analysis of confocal microscopy images revealed that neither chronic restraint stress nor LSI affect the total number of PV cells in the BLA (2-way ANOVA, main effect of drink = 0.1414, main effect of stress = 0.7290, drink X stress interaction = 0.7855). Similarly, neither stress nor LSI affect total integrated density of PNNs in the BLA (2-way ANOVA, main effect of drink = 0.291, main effect of stress = 0.718, drink X stress interaction = 0.722). However, LSI significantly increases the proportion of BLA PV neurons that have PNNs while repeated restraint stress tends to have an opposite effect (2-way ANOVA, main effect of drink = 0.009, main effect of stress = 0.06, drink X stress interaction = 0.952). This suggests that LSI decreases PV interneuron structural plasticity and hence impedes the formation of synapses with other GABAergic inputs, reducing overall BLA output to stress-related brain regions, a possibility that future studies will address. The results of this line of investigation will further our understanding of the neurological circuitry regulating stress and reward and will inform the development of therapies that integrate naturally rewarding experiences in the management of stress-related disorders.

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Poster

646. Stress-Modulated Pathways: Hypothalamus and Bed Nucleus

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 646.20

Topic: F.03. Stress and the Brain

Support: R01 DK118292

Title: Brain cannabinoid receptor 1 signaling participates to stress relief by comfort foods

Authors: *K. ALMEHMADI[1], D. BUSING[2], A. SABO[2], A. KING[2], Y. ULRICH-LAI[2]; [2]Pharmacol. and system Physiol., [1]Univ. of Cincinnati, Cincinnati, OH

Abstract: Rationale: Many individuals report that eating tasty food reduces their stress. However, the mechanisms underlying stress relief by palatable foods are unclear. Endocannabinoid signaling at the cannabinoid receptor 1 (CB1R) in the brain promotes feeding and reduces hypothalamic-pituitary-adrenocortical (HPA) axis responses to stress, placing it in a prime position to mediate palatable food-induced-stress relief. Here we test the hypothesis that brain CB1R signaling mediates the reduced HPA axis responsivity observed following an established limited sucrose intake (LSI) paradigm in rats. Methods: Adult male rats received stereotaxic surgery to implant a cannula directed to the lateral ventricle. Following recovery, rats with free access to chow and water were given limited access to an additional drink bottle containing either a small amount (4 ml) of 30% sucrose solution or water (controls) twice daily for 14 days. On day 15, rats from each drink group received an intracerebroventricular (icv) infusion of either AM251 (CB1R inverse agonist, 1000 ng/2 ul) or vehicle (n =13-17/group). Following a 30-min resting period, the rats were given a 20-min restraint stress and tail blood samples were collected at 0, 20, 40 and 60 min after the onset of restraint, for the purpose of measuring plasma corticosterone levels as an indicator of the stress (HPA axis) response. At the end of the experiment, rats received an icv infusion of toluidine blue prior to brain collection to confirm correct cannula placement and patency. Results: Plasma corticosterone responses to restraint stress was reduced in the LSI group compared to water controls (p=0.004). Post-hoc analyses indicated that this effect occurred primarily in the vehicle-treated groups (positive control for effective LSI HPA-blunting), and not in the AM251-treated groups. Taken together, the data implicate brain CB1R signaling as a mechanism for LSI stress relief.

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Poster

647. Neuroimmune Function, Activation, and Regulation

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 647.01

Topic: F.04. Neuroimmunology

Title: Type I interferon alters the metabolic signaling and neuronal activities in hypothalamic POMC neurons

Authors: Y.-S. LIM[1], Y.-C. LIAO[2], P.-W. CHU[2], ***S.-K. CHEN**[1];
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Abstract: Accumulating data have suggested that pro-inflammatory cytokines can act as neuromodulators affecting neural physiology. Type I interferon, including interferon alpha and beta, are primary anti-viral effectors of the innate immune system, and have been clinically employed for treating viral and autoimmune diseases. However, chronic treatment of type I interferon also leads to a wide range of side effects, including neurological defects. The

depressive symptoms and decrease of food intake have been shown to be associated with neurological dysfunctions. In this study, we aimed to investigate the effects of type I interferon on neural functions and activities. Owing to the critical roles of hypothalamic POMC neurons in regulating feeding behavior, we examined the cellular responses of mHypoA-POMC/GFP1 to the interferon treatment. This immortalized cell line has been previously proven that key metabolic responses and neuronal characteristics of hypothalamic POMC neurons are maintained and can be used to mimic the hypothalamic neuron functions. Interferon signaling, including the expression of STATs and downstream genes such as *socs* genes, were upregulated, implying that interferon signaling and might be physiologically effective in these neurons. Our previous data showed that the activities of these neurons, which are determined by measuring the expression of immediate early gene *c-fos*, remained low under the unstimulated conditions, but was elevated by metabolic signals such as insulin and leptin. Interferon treatment does not alter neuronal activities, but disrupted the insulin-induced neuronal activation. Interestingly, the effects of interferon signaling on neural activity appeared to be time-dependent, suppressing neural activity during the first three hours of the exposure. The inhibitory effects were reversed afterward. Also, previous report demonstrated that STING-induced interferon signaling activates sensory neurons. Our data revealed different responses of hypothalamic neurons.

Disclosures: Y. Lim: None. **Y. Liao:** None. **P. Chu:** None. **S. Chen:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 647.02

Topic: F.04. Neuroimmunology

Support: Anschutz Pandemic Preparedness Grant 6476340

Title: Calcitonin Gene Related Peptide (CGRP) Modulates B cells in the Lung Neuroimmune Axis

Authors: *B. PATLIN[1], L. SCHWERDTFEGER[3], S. TOBET[2];
[1]Colorado State Univ., [2]Colorado State Univ., Fort Collins, CO; [3]Harvard Univ., Cambridge, MA

Abstract: The role of neural inputs to the lung are often overlooked despite the numerous neurotransmitters that are in the lung—from resident neurons to peripheral nervous system fibers. To test the impact of neural-immune interactions, a murine organotypic lung slice model was developed and characterized over 6 days ex vivo using adult serum-free neurobasal-CTS media +B27 supplement. In vivo and ex vivo conditions were assessed in drop fixed lung sections compared to cultured slices, respectively, by immunohistochemistry. Lung slices up to 6 days ex vivo demonstrated comparable levels of $CD19^+$ (immunoreactive) B cells, surfactant C^+ alveolar type 2 cells, calcitonin gene related peptide$^+$ ($CGRP^+$) cells and fibers, vasoactive intestinal peptide$^+$ fibers, substance P^+ fibers, and peripherin$^+$ fibers compared to in vivo sections.

Mucus production was assessed by incorporation of a modified galactosamine (GalNAz) with visualization through click-chemistry (Schwerdtfeger & Tobet, Physiol Rep 2020;8:e14363). Cell proliferation was estimated ex vivo by incorporation of 5-Ethynyl-2′-deoxyuridine (EdU) and was comparable to expected ranges of proliferation in non-injured lung tissue. Cell death was assessed using acridine orange and was minimal and constant over 6 days ex vivo. Since $CGRP^+$ fibers and cells were the most prevalent of the neuronal elements, slices were exposed to 10μM CGRP in culture media for 24- or 48h and assessed for immune responses. $CD19^+$ B cell numbers increased and their distribution throughout the slices appeared more dispersed over 24 to 48h after CGRP treatment. B cells are located primarily in vivo and ex vivo in bronchiolar associated lymphoid tissues at airway-vasculature junctions under normal culture conditions and when not infected. After exposure to CGRP, B cells were spread further across alveolar, bronchiolar, and pleural spaces. The pattern of surfactant C^+ cells in alveoli changed following CGRP exposure suggesting a potential impact on epithelial cell function. The number of visible $CGRP^+$ fibers decreased while the number of $peripherin^+$ fibers did not appear to change. The loss of immunoreactive CGRP may indicate endogenous CGRP release. CGRP-induced changes in the B-cell landscape may have implications for pulmonary disease treatment and indicate a role for the nervous system in response to peripheral infections.

Disclosures: B. Patlin: None. **L. schwerdtfeger:** None. **S. Tobet:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

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Program #/Poster #: 647.03

Topic: F.04. Neuroimmunology

Support: 2R01MH052716-26
F30HD107942-01A1

Title: Altering stem cell factor and c-kit signaling impacts the proliferation of hippocampal mast cells in early postnatal development

Authors: *A. A. MAXIMOVA, A. C. BLANCHARD, M. M. MCCARTHY;
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Abstract: Inflammation in the neonatal brain has been firmly linked to the progression of neuropsychiatric diseases. Less well understood is the role the immune system normally plays during critical periods in brain development. Of particular interest are mast cells (MCs), an innate immune cell that releases a myriad of substances such as histamine, cytokines, and growth factors in the setting of host defense, allergy, or inflammation. In addition to their classic niches in connective tissue and mucosal barriers, MCs are also found in distinct populations in the developing brain. Previous work from this lab established MC activation in the preoptic area (POA) during a critical period is sufficient to alter synaptic patterning and adult behavior in a rat

model. We now report on a robust MC population lining the ventricles next to the developing hippocampus, which peaks in cell number at postnatal (PN) day 7, declines during week 2, and is absent into adulthood. This population is also highly proliferative, a key difference from POA MCs and unlike other resident peripheral MCs, excluding the bone marrow. This project seeks to understand factors regulating this difference in proliferative capacity by studying c-kit, a receptor tyrosine kinase, and its ligand, stem cell factor (SCF), both of which regulate peripheral MC proliferation. We hypothesize that a higher degree of SCF is expressed before the peak of hippocampal MCs, and that blocking either the SCF ligand or the c-kit receptor will reduce their proliferation. We began by characterizing expression of both SCF and c-kit across the first few weeks of life via RT-qPCR. Preliminary results reveal an increase in SCF expression in the hippocampus at PN4 and decrease after PN8/PN12 in males only, displaying an unexpected sex difference that will be investigated further. Next, to test the effectiveness of SCF and c-kit blocking antibodies, we cultured MCs from homogenized PN0 rat hippocampus and bone marrow using media supplemented with IL-3 and SCF. Addition of SCF or c-kit blocking antibodies caused a 37-90% reduction in MC proliferation from the hippocampus-derived population, as measured by both BrdU and EdU incorporation. Data collection is ongoing for SCF/c-kit blocking in vivo, as well as studying if adding exogenous SCF alters the timing of the MC peak. Further studies will use SCF/c-kit signaling as a tool to manipulate MCs and study their impact on hippocampal-mediated behavior. These studies will lay the groundwork for exploring how mast cells shape early postnatal development and help establish the importance of innate immune cells in the developing brain.

Disclosures: **A.A. Maximova:** None. **A.C. Blanchard:** None. **M.M. McCarthy:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

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Program #/Poster #: 647.04

Topic: F.04. Neuroimmunology

Support: CNPq
CAPES
FAPERJ

Title: Temporal dynamics of lymphocyte distribution in the brain during postnatal development

Authors: *D. P. DANTAS, L. C. R. MAIA, R. MENDEZ-OTERO, A. M. VALE, P. M. PIMENTEL-COELHO;
Inst. de Biofísica Carlos Chagas Filho, Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: The concept of brain immunoprivilege has been revised with the findings of the presence of lymphocytes and their possible functions in the brain. Furthermore, the characterization of the lymphopoietic niche located in the cerebral meninges has raised interest

in the importance of these cells for brain functioning and immunosurveillance. However, little is known about how and when lymphocytes begin to populate the brain.
The aim of this study was to characterize the presence of B and T lymphocyte cells during murine brain development and to identify the location of cells in relation to blood vessels. C57BL/6 mice aged P0 (day of birth), P3, and P14 were used, as well as 3-month-old young adult animals of both sexes. The brains were analyzed by immunofluorescence analysis and confocal microscopy. Flow cytometry was used to further characterize the lymphocyte populations in a BD LSR Fortessa flow cytometer. All experiments were approved by the Animal Ethics Committee of our Institution (protocol CEUA/CCS-UFRJ 080/17).Antibodies to CD3 and CD45R were used to identify T and B cells, respectively, in different regions of the mouse brain, including leptomeninges, neocortex, hippocampus, striatum, thalamus and cerebellum. B cells are present since P0 until adulthood when their numbers decreases significantly in all analyzed regions. In contrast, there are fewer T cells during early postnatal development in comparison to the adult brain. Using laminin as a marker of blood vessels, we found T and B cells associated with the vessels, but also some T cells in the brain parenchyma. Flow cytometry results are in progress.Lymphocyte distribution is dynamic during brain development and essentially associated with blood vessels in different brain regions.

Disclosures: **D.P. Dantas:** None. **L.C.R. Maia:** None. **R. Mendez-Otero:** None. **A.M. Vale:** None. **P.M. Pimentel-Coelho:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

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Program #/Poster #: 647.05

Topic: F.04. Neuroimmunology

Support: R01CA194924
U54GM104942

Title: Social Enrichment Enhances Chemotherapy Induced Monocyte Trafficking in Aged Mice

Authors: *W. H. WALKER, II[1], L. E. MAY[1], J. A. LIU[1], R. J. NELSON[1], A. DEVRIES[2]; [1]Neurosci., [2]Med., West Virginia Univ., Morgantown, WV

Abstract: Chemotherapy is a mainstay in cancer treatment and has demonstrated superior anti-tumor efficacy for most cancers. However, chemotherapy treatment is not without adverse side effects. Patients receiving chemotherapy regularly report altered fatigue, sleep, cognitive function, and depression. Notably, the amount of social support prior to cancer treatment predicts the levels of cognitive dysfunction and depressive symptoms in cancer survivors. Previous work from our lab, demonstrates that social enrichment attenuates chemotherapy induced pro-inflammatory cytokine production and affective behavior in adult female mice. However, the effect of social enrichment and mechanism(s) underlying chemotherapy-induced CNS changes in

aged mice remains unknown. We hypothesized that chemotherapy increases peripheral immune cell trafficking in aged mice and predicted that social enrichment would attenuate this effect. Aged (~20 months) female Balb/C mice were singly or grouped housed (with two ovariectomized females) and received two injections, separated by two weeks, of vehicle or a chemotherapeutic cocktail (9 mg/kg doxorubicin and 90 mg/kg cyclophosphamide). Seven days following the second chemotherapy injection, mice were euthanized via intracardiac perfusion, brains were extracted, and immune cells were isolated via Percoll gradient and quantified by flow cytometry. Chemotherapy treatment did not alter the percentage of microglia, activated microglia, or granulocytes within the brain. Additionally, chemotherapy had no effect on microglia or granulocyte cytokine production. However, chemotherapy significantly increased monocyte trafficking within the brain. Social enrichment increased monocyte trafficking ($CD11b^{+}CD45^{High}$ $Ly6C^{-}Ly6G^{-}$) relative to singly and grouped housed vehicle treated mice, an effect not seen in singly housed mice receiving chemotherapy. Specifically, social enrichment increased IL-1^{+} monocytes. Next, we sought to determine the functional effects of social housing in aged mice in a more translational paradigm. In addition, to the methods outlined above, aged female Balb/C mice received bilateral orthotopic injections of 67NR cells ($1x10^{5}$ cells/injection) one week prior to their first chemotherapy injection. Social enrichment in aged mice did not alter tumor growth or the response to chemotherapy. In addition, housing status did not alter corticosterone. Future studies will seek to address the age of partners and timing of social enrichment to further delineate the effects of social housing on tumor growth in aged mice.

Disclosures: W.H. Walker: None. **L.E. May:** None. **J.A. Liu:** None. **R.J. Nelson:** None. **A. DeVries:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

Location: SDCC Halls B-H

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Program #/Poster #: 647.06

Topic: F.04. Neuroimmunology

Title: Neuroinflammatory responses following open flow microdialysis

Authors: *T. KIM, C. CHAU, I. CHENG, S. YELLAI;
Charles River Labs., South San Francisco, CA

Abstract: Open Flow Microdialysis isa technique used to recover molecules of high molecular mass, such as peptidesand proteins, from the interstitial space in the CNS. For this, a membrane-freeprobe is implanted and perfused to obtain a diluted but unfiltered interstitialfluid sample. However, there is evidence to suggest that CNS implants caninduce a neuroinflammatory response, including glial activation and increase inpro-inflammatory cytokine levels. This study investigates the potentialneuroinflammatory effects of open flow microdialysis (OFMD). To do so, twelvefemale Sprague Dawley rats were implanted with cOFM guide canula and healing dummy in the striatum. After two weeks recovery time, six of the ratsunderwent OFMD. cOFM

probes were inserted and connected to inlet and outletlines and allowed to stabilize for one hour. After stabilization, dialysatesamples were collected for an additional three hours at 1-hour intervals. Theother six animals served as naïve controls and did not undergo OFMD sampling.At the end of sampling, plasma was collected via lateral tail vein, andterminal CSF, brain, liver, spleen, and spinal cord were extracted from all theanimals for analysis. All samples will be analyzed via MSD® V-PLEX Proinflammatory Panel 1 Mouse kits(Cat No. K15048D) for the following cytokines: IFN-γ, IL-12p70, IL-1β, IL-2,IL-4, IL-5, IL-6, IL-10, KC/GRO, and TNF-α. We expect to find increased levelsof cytokines from the CSF and brain samples collected from the animals thatunderwent microdialysis as compared to the naïve controls. We do not expect tofind cytokines in the liver or spleen from either naïve or microdialysisanimals. We hope to gain a greater understanding of the potential inflammatoryand immunological effects of OFMD at the cellular and molecular level from thisstudy.

Disclosures: T. Kim: None. **C. Chau:** None. **I. Cheng:** None. **S. Yellai:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 647.07

Topic: F.04. Neuroimmunology

Title: Neuroimmune activation of the olfactory bulb is regulated by time of day

Authors: *G. PEARSON, B. FALCY, J. WANG, S. AKLI, I. KARATSOREOS;
Neurosci. and Behavior Program, Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Background: Given its proximity to the nasal cavity, the olfactory bulb (OB) must generate robust neuroimmune responses to defend against neurotropic pathogens. The circadian clock primes cells and tissues to anticipate physiologically relevant environmental changes. We hypothesized that daily changes in OB neuroinflammatory state would differentially "prime" responses to an intranasal inflammatory challenge. Aim 1 probed the OB's neuroinflammatory transcriptional profile throughout the day. Aim 2 investigated how time of day of an intranasal inflammatory challenge influences the cellular responses within the OB. **Methods:** For Aim 1, OBs were isolated from male C57BL/6N mice at 8 times of day (n = 3 mice/time, N = 24, 11-13 weeks old). Transcriptional profiles of OBs were assessed using a NanoString Murine nCounter Neuroinflammation Panel. Gene expression data were normalized to housekeeping genes using Rosalind software and assessed for rhythmic expression using JTK cycle analysis. For Aim 2, we intranasally challenged male C57BL/6N mice (n = 4 mice/time/treatment, N = 24, 15-18 weeks old) at different times of day with poly(I:C) (dosages of 10 μg or 20 μg, 10 μL per nare) or a vehicle control and then measured cellular responses within the OB at 24-hours post-inoculation using imaging flow cytometry. Intranasal challenge experiments were completed over three independent experiments, with each experiment consisting of a single treatment at two times of day. Compensation and fluorescence minus one (FMO) controls were used for compensating

flow cytometry data and confirming positive populations, respectively. To assess the effect of time of day and treatment a two-way ANOVA was used. **Results:** We identified 154/757 neuroinflammatory-related genes (20%) that were rhythmically expressed (JTK cycle analysis, adj. $p < 0.05$) in the OB under baseline conditions. These rhythmically-expressed genes were enriched in the type I interferon signaling pathway with elevated expression at the beginning of the active phase. We also observed rhythmic expression of genes involved in microglia function including several involved in sensing changes in brain state. Following intranasal challenge with poly(I:C), we observed enhanced ability by OB microglia ($CD11b^{+}$, $CD45^{low}$, $P2RY12^{+}$ cells) to fluctuate the surface expression of CD11b at ZT12 compared to ZT0. **Conclusion:** The circadian clock may prime the OB's response to intranasal inflammatory stimuli differently depending on time of day of exposure, providing a potential gating mechanism underlying differential susceptibility to pathogen exposure via the nasal route, including neurotrophic pathogens.

Disclosures: G. Pearson: None. **B. Falcy:** None. **J. Wang:** None. **S. Akli:** None. **I. Karatsoreos:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

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Program #/Poster #: 647.08

Topic: F.04. Neuroimmunology

Support: 5R01NS092388
5U54GM104942

Title: Dim Light at Night Increases Microglial Activation Post Stroke

Authors: *J. A. LIU, W. H. WALKER, II, O. H. MELÉNDEZ-FERNÁNDEZ, J. R. BUMGARNER, N. ZHANG, A. C. DEVRIES, R. NELSON;
West Virginia Univ., West Virginia Univ., Morgantown, WV

Abstract: Light at night (LAN) disrupts circadian clock rhythmicity and alters several aspects of immune function, including dysregulating pro-inflammatory cytokine production and altering aspects of the innate and adaptive immune system in otherwise healthy animals. Previous research on the effects of dim LAN (dLAN) exposure on ischemic injury outcomes in a murine model of ischemic stroke revealed that dLAN exacerbated infarct size, sensorimotor deficits, and reduced survival across 24 h. To examine the role of microglia in infarct progression we hypothesized that exposure to dim light at night increases pro-inflammatory microglia that amplifies inflammatory response post stroke. Adult mice received a transient right middle artery occlusion (MCAO) for 45 minutes, then were placed into a single dark night (LD 12 h light 150 lux; 12 h dark 0 lux) or dLAN (dark - 5 lux) conditions. 24 hours post stroke, brains were collected and flow cytometry was performed to analyze any changes in immune cell populations. We observed no differences in the total percentage of CD11b+ microglia between lighting

conditions, but instead observed increased activated microglia that express MHC-II and IL-6 in mice housed in dLAN post stroke compared to dark night conditions, suggesting that nighttime lighting alters brain-immune interaction exacerbating stroke outcome. Next, a CSFR1 inhibitor, Plexxikon 5622 (1200 ppm), was administered 7 days prior to stroke to selectively eliminate microglia in the brain, and we repeated the experimental design. We observed that elimination of microglia normalizes infarct size between lighting conditions, suggesting that microglia are responsible for exacerbating injury during acute circadian disruption. dLAN can be especially detrimental in compromised health states such as stroke, and our results, if translatable to humans, suggest that reducing or altering disruptions of circadian rhythms could improve patient outcome.

Disclosures: **J.A. Liu:** None. **W.H. Walker:** None. **O.H. Meléndez-Fernández:** None. **J.R. Bumgarner:** None. **N. Zhang:** None. **A.C. DeVries:** None. **R. Nelson:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 647.09

Topic: F.04. Neuroimmunology

Support: DFG Grant 316803389

Title: Effects of taste-immune associative learning on experimentally induced glioblastoma in rats

Authors: S. HETZE[1], L. BARTHEL[1], H. S. GÜNTHER[3], C. WÜLFING[3], M. SCHEDLOWSKI[4], ***M. HADAMITZKY**[2];
[2]Univ. Hosp. Essen, [1]Univ. Hosp. Essen, Essen, Germany; [3]Univ. of Hamburg, Hamburg, Germany; [4]Univ. Clin. Essen, Univ. Clin. Essen, Essen, Germany

Abstract: Mechanistic target of rapamycin (mTOR)-signaling is one key driver in glioblastoma (GBM) tumor growth by promoting the shift to an anti-inflammatory, pro-cancerogenic microenvironment. In fact, mTOR inhibitors such as rapamycin have been shown to interfere with GBM disease progression, however, therapy in both animal models and patients is frequently chaperoned by toxic drug side effects. Since recent findings document that taste-immune associative learning with rapamycin induces pharmacological placebo responses in the immune system, the present report analyzed its applicability in a syngeneic GBM rat model. Following repeated pairings of a novel gustatory stimulus with injections of rapamycin, learned immunopharmacological effects were retrieved in GBM-bearing animals when re-exposed to the gustatory stimulus together with administering just 10% of the therapeutic drug dose. This procedure promoted a pro-inflammatory anti-tumor microenvironment and effectively prevented tumor growth to an almost identical outcome obtained after full dose treatment. Collectively, this proof-of-concept study document that taste-immune associative learning strategies may be

utilized as supportive treatment strategy, allowing the reduction of required drug doses and side effects without losing treatment efficacy. This work was funded by center grants of the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) project number 316803389 - SFB 1280 (TP A18 -19/1-2).

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Poster

647. Neuroimmune Function, Activation, and Regulation

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Topic: F.04. Neuroimmunology

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Korea NRF Grant 2022M3E5E8030792

Title: The control of IL21 in hypothalamus reduces metabolic disfunction

Authors: *M. KIM, J. KIM, Y. LEE;
Brain Sci. Inst., Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: IL21 released by damage- or pathogen- associated molecularpatterns is substantial for adaptive immunity. It is well-known for its immuneactivities but a role of hypothalamic IL21 is unclear. Here, we investigateneural activities of hypothalamic IL21 in a metabolic disorder, such as anobesity. We observed significantly high expression of basal hypothalamic IL21levels in an obese condition. For further investigation, we madeadeno-associated viral (AAV) vectors expressing short hairpin RNAs (shRNA)targeting IL21 mRNA (AAV-shRNA-IL21) or control AAV-scrambled-shRNA then theywere bilaterally injected to medio-basal hypothalamus of wild type C57BL/6mice. These mice were fed a chow diet or high-fat diet for three months. Wemeasured that these mice's body weights, glucose tolerance, metabolic rates andmotor activities. The mice were sacrificed after and behavioral test and allphysiological for analyzing molecular signaling of hypothalamus. The inhibitionof hypothalamic IL21 reduced body weights and glucose levels in an obesecondition. Interestingly, these mice presented significantly higher physiologicalactivities, compare to control groups. Finally, we found that hypothalamic IL21induction mediated the brain's action in promoting the increase activity toimprove metabolic syndrome.

Disclosures: M. Kim: None. **J. Kim:** None. **Y. Lee:** None.

Poster

647. Neuroimmune Function, Activation, and Regulation

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Topic: F.04. Neuroimmunology

Support: NIH Grant NS101541
NIH Grant NS127364

Title: Dietary lipids suppress developmental and behavioral phenotypes of hyperexcitable Drosophila mutants: a potential involvement of neuroimmune interactions

Authors: J. KASUYA[1], W. JOHNSON[2], *T. KITAMOTO[3];
[1]Neurosci. and Pharmacol., [2]Mol. Physiol. and Biophysics, [3]Anesthesia, Univ. of Iowa, IOWA CITY, IA

Abstract: Dietary modifications often have a profound impact on the penetrance and expressivity of neurological phenotypes caused by genetic defects. Our previous studies in *Drosophila* revealed that seizure-like phenotypes of voltage-gated sodium (Na_v) channel mutants, including $para^{Shu}$, were drastically suppressed by supplementation of a standard diet with milk whey (Kasuya et al., 2019). In the current study, through physical and chemical fractionation analyses, we find that supplementing the diet with a modest amount of milk lipids (0.26% w/v) mimics the seizure-suppressing effects of the milk whey diet. Since the dietary modification only during the larval stages effectively suppresses adult $para^{Shu}$ phenotypes, dietary lipids most likely modify neural development to compensate for the defects caused by the mutations. Consistent with this notion, we show that class IV sensory neurons in $para^{Shu}$ larvae display significantly greater dendritic complexity compared with the control larvae, and that lipid feeding fully rescues this developmental phenotype of the mutant. To gain insights into the mechanisms underlying the diet-dependent suppression of seizures, we screened for second-site mutations that mimic the effects of dietary lipids on $para^{Shu}$ hyperexcitability. One of the genes identified in this genetic screen is *glutathione S-transferase S1* (*GstS1*), a putative ortholog of mammalian prostaglandin D2 synthase (Chen et al., 2020). Interestingly, we demonstrate that the severity of $para^{Shu}$ phenotypes is reduced by RNAi-induced knockdown of *GstS1* expression in blood cells, which are responsible for cellular innate immunity. Furthermore, we found that a minor milk lipid component, alpha-linolenic acid (ALA), contributes to the diet-dependent suppression of adult $para^{Shu}$ phenotypes. In mammals, ALA is involved in synthesis of potent bioactive lipid mediators such as prostaglandins, and play important roles in optimal functioning of the immune system. Taken together, our results raise the intriguing possibility that dietary lipids lead to suppression of hyperexcitable phenotypes of seizure-prone *Drosophila* mutants by modifying neural properties through interactions between the developing nervous system with lipid-mediated innate immune signaling.

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Poster

647. Neuroimmune Function, Activation, and Regulation

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Program #/Poster #: 647.12

Topic: F.04. Neuroimmunology

Support: R01GM128008

Title: Differential regulation of inflammation by the brainstem dorsal motor nucleus of the vagus and nucleus ambiguus

Authors: *A. FALVEY[1], S. P. PALANDIRA[1,2], K. J. TRACEY[1,2,3], V. A. PAVLOV[1,2,3];
[1]The Feinstein Inst. for Med. Res., Manhasset, NY; [2]Elmezzi Grad. Sch. of Mol. Med., Manhasset, NY; [3]Donald and Barbara Zucker Sch. of Med. at Hofstra/Northwell, Manhasset, NY

Abstract: The vagus nerve regulates physiological processes including immunity and inflammation. The brainstem dorsal motor nucleus of the vagus (DMN) and nucleus ambiguus (NA) supply the motor (efferent) cholinergic neurons in the vagus nerve. Optogenetic DMN stimulation was recently shown to mitigate serum TNF levels during endotoxemia. However, the immunoregulatory effects of electrical DMN stimulation, which has a broad current use in the new field of bioelectronic medicine, remained unknown. The contribution of the NA to the vagus nerve inflammatory regulation also remained enigmatic. To provide insight, we investigated the effects of DMN electrical stimulation and NA electrical and optogenetic stimulation in mice with two inflammatory conditions - endotoxemia and cecal ligation and puncture (CLP)-induced polymicrobial sepsis. DMN electrical stimulation was performed in anesthetized C57Bl/6 male mice using a stereotaxic frame. A concentric bipolar electrode was guided to the coordinates of the left DMN. Electrical stimulation (or sham stimulation) was performed for 5 mins and LPS (0.5 mg/kg) was injected (i.p.). 90 mins later blood and spleen were collected and processed for cytokine analyses. CLP was induced using a standardized procedure prior to electrical or sham DMN stimulation (as described above) and blood and spleen were collected 24h later. Left NA optogenetic (in ChAT-ChR2-eYFP) or electrical (in C57BL/6) stimulation in male mice was performed using a stereotaxic guidance prior to LPS administration and blood and spleen were collected for cytokine analysis. Electrical DMN stimulation significantly reduced pro-inflammatory cytokine levels in the serum (TNF: $p = 0.0012$; IL-6: $p = 0.427$) and spleen (TNF: $p = 0.0001$; IL-6: $p = 0.0077$), as well as increased the serum levels of the anti-inflammatory cytokine IL-10 ($p = 0.0284$) during endotoxemia. In animals subjected to CLP, electrical DMN stimulation significantly decreased pro-inflammatory cytokines in the serum (TNF: $p = 0.0291$; IL-6: $p = 0.0332$) and spleen (IL-6: $p = 0.019$). In contrast, neither optogenetic nor electrical NA stimulation altered serum and spleen cytokine levels during endotoxemia. In conclusion, these results indicate that while electrical DMN stimulation alters cytokine levels in endotoxemia and in CLP (a clinically relevant model of sepsis), stimulation of the NA (optogenetic or electrical) does not have significant effects. These findings identify the brainstem DMN as a major source of efferent vagal neurons controlling cytokine responses and inflammation. They are of interest for novel therapeutic strategies focusing on the DMN in the treatment of inflammatory conditions.

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Poster

647. Neuroimmune Function, Activation, and Regulation

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Program #/Poster #: 647.13

Topic: F.04. Neuroimmunology

Support: R01GM143362

Title: Imaging and analysis of dynamic neuronal activity in the jugular-nodose ganglia of mice

Authors: *T. S. HUERTA[1], B. HAIDER[1], R. ADAMOVICH-ZEITLIN[1], A. C. CHEN[1], S. CHAUDHRY[1], S. S. CHAVAN[2], K. J. TRACEY[3], E. H. CHANG[2];
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Abstract: Neuron cell bodies of the peripheral nervous system (PNS) reside within ganglia containing thousands of cells, often residing in regions difficult to access and image. It is challenging to assess the structure and function *In vivo* of peripheral nerves at the single neuron level. Micro-endoscopic calcium imaging is an optophysiological approach to record dynamic neural activity across large populations of neurons, but its application has been limited to brain neurons, not PNS targets. Here we develop a novel method for imaging the intact jugular-nodose ganglion of the PNS using a miniature microscope (Miniscope) in a transgenic mouse with the genetically encoded calcium indicator GCaMP6f in glutamatergic sensory neurons expressing VGlut2-cre. To image the jugular-nodose ganglion, the vagus nerve was surgically exposed and the jugular-nodose complex stabilized on a mesh retractor, while carefully preserving the neural projections to the peripheral organs. We adapted the Python-based analysis tool CaImAn to process the resulting one-photon florescence data from detected cell soma into calcium transients for subsequent analysis. This acquisition and analysis strategy was used to successfully image the jugular-nodose ganglia in VGlut2-GCaMP6f mice during application of capsaicin and glutamate. Neural responses to stimuli were analyzed by response amplitudes and durations, and cell-specific responses quantified. We observed that capsaicin induced significantly higher peak amplitude as compared to glutamate peak amplitudes (dF/F, 69.30 ± 11.02 vs 34.50 ± 4.126, $p < 0.01$, Mann-Whitney U test), suggesting capsaicin mediates higher calcium flux as compared to glutamate. The duration of average capsaicin responses was significantly shorter as compared to glutamate (msec, 179.8 ± 25.48 vs 331.4 ± 45.8, $p < 0.05$, Mann-Whitney U test), indicating that glutamate mediates a significantly more prolonged calcium flux. Thus, a novel surgical and analytical approach can be applied to dynamic large-scale imaging of small peripheral ganglia in the murine nodose ganglia. Open-sourced solutions, such as the Miniscope and CaImAn, should allow researchers to customize their own pipelines for the acquisition and analysis of calcium imaging datasets for other targets of interest.

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Poster

647. Neuroimmune Function, Activation, and Regulation

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 647.14

Topic: F.04. Neuroimmunology

Support: NIH Grant R01GM132672
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Title: Specific neuronal populations encode inflammatory responses

Authors: *O. HASHIMOTO[1], T. HEPLER[1], T. TSAAVA[1], A. TYNAN[1], K. J. TRACEY[1,2], S. S. CHAVAN[1,2];
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Abstract: The brain maintains a homeostatic condition in the body through neuronal communication with peripheral organs. The immune system is also regulated by the nervous system with principles of reflex regulation. Neuronal pathways, including the vagus nerve-based inflammatory reflex, are physiological regulators of immune function and inflammation. However, it remains unclear whether and how the brain encodes the state of the immune system. We previously showed that the projections from the brainstem dorsal motor nucleus of the vagus nerve control inflammatory responses in the spleen. Furthermore, we found that cytokine-specific information is present in sensory neural signals within the vagus nerve. Based on these findings, we reasoned that the brain encodes and stores cytokine-specific information. Here, we carried out activity-dependent cell labeling in mice. We utilized the targeted-recombination-in-active-populations (TRAP2) mice crossed with a tdTomato reporter line to produce double transgenic TRAP2/tdTomato mice. We captured neuronal ensembles that were active in response to tumor necrosis factor (TNF) or interleukin-1β (IL-1β) administration. Increased tdTomato expression (indicative of neuronal activity) in response to TNF or IL-1β administration is observed in the paraventricular nucleus (PVN), the bed nuclei of the stria terminalis (BNST), and the nucleus of the solitary tract (NTS), where vagal afferents ascending from peripheral tissues synapse on neurons. Re-exposure of mice to either TNF or IL-1β results in the labeling of additional neuronal populations in the BNST. A significant population of TNF-responsive neurons is found to be localized in the protein kinase C delta (PKCδ)-positive subnucleus within the BNST, while IL-1β-responsive tdTomato marked neurons are predominantly located in the PKCδ-negative region within the BNST. These results suggest that the brain encodes the TNF and IL-1β-specific information in distinct regions.

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Poster

647. Neuroimmune Function, Activation, and Regulation

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Program #/Poster #: 647.15

Topic: F.04. Neuroimmunology

Support: NIH grant K22NS096030
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Title: The role of macrophages in ovarian turnover and fertility

Authors: *H. M. ABDELHADI, M. E. LENERT, M. D. BURTON;
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Abstract: In the United States, approximately 15% of women face infertility. Inflammation plays a vital role in ovarian function, however, the underlying immune mechanisms have yet to be elucidated. We are interested in tissue-resident macrophages, one of the critical local immune cells that remove cell debris, controls inflammation, and initiates tissue repair. Shifts in macrophage reactivity are important for homeostasis and inflammation and are regulated by the cell's metabolic state. In our study, we investigated how macrophage metabolic state regulates inflammation, tissue repair, ovarian turnover, and fertility. The Liver Kinase B1 (LKB1) pathway is involved in maintaining metabolic homeostasis and is activated during metabolic stress. Previous literature shows that the LKB1 pathway drives an anti-inflammatory/tissue repair state in macrophages. We hypothesized that LKB1-positive/anti-inflammatory macrophages are important for ovarian turnover and normal fertility. Our study used transgenic mice with LKB1 removed from macrophages, as well as their cre negative (wildtype) counterparts as controls. Female breeders with LKB1 removed from macrophages have both significantly smaller litters and a greater number of follicles compared to wildtype females; these findings suggest that ovarian turnover is delayed. We performed immunohistochemical staining for active macrophages in the ovaries across the estrus cycle and found more macrophages in the ovary during the later (post-ovulatory) phase of animals with LKB1 removed from their macrophages. We believe, anti-inflammatory macrophages facilitate the breakdown and recycling of structures during the luteal phase, suggesting a vital role in ovarian turnover. We are also assessing metabolic output from these macrophages. We believe investigating the role that macrophage activation plays in regulating ovarian function can lead to novel therapeutics for improving fertility outcomes.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 648.01

Title: WITHDRAWN

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 648.02

Topic: F.08. Food and Water Intake and Energy Balance

Support: 25210-49000-424001-516468

Title: Short-term High Fat Diet Impairs Hippocampal Function and Responsiveness to Feeding Status

Authors: *T. LANDRY[1], S. TART[2], L. ZHANG[1], J. SONG[1];
[1]Pharmacol., [2]Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Short-term High Fat Diet Impairs Hippocampal Function and Responsiveness to Feeding Status Taylor Landry[1], Seth Tart[1], Libo Zhang[1], & Juan Song[1,2]1.*Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*2.*Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.*
Epidemiological studies demonstrate metabolic diseases such as diabetes and obesity are associated with increased incidence of cognitive decline. However, the mechanistic link between these diseases and cognitive dysfunction remains unclear. Rodent models of metabolic disease exhibit impaired hippocampal neurogenesis, decreased synaptic complexity, and increased apoptosis in the dentate gyrus (DG) of the hippocampus, a critical brain region involved in cognitive function. Therefore, we hypothesize that DG function is finely regulated by whole-body energy status, and becomes dysregulated in response to high fat diet (HFD), leading to cognitive deficits. Using immunohistochemistry in the DG of healthy mice, we observed decreased neural progenitor proliferation and reduced cFos expression in granule cells (GC) in response to fasting, which were robustly increased after refeeding. Similarly, *in vivo* fiber photometry revealed long-lasting increases in GC activity after refeeding, while GABAergic interneurons experienced opposite effects. These effects were also observed in response to an intraperitoneal (IP) glucose injection. Interestingly, just 5 days of HFD blunted the GC response to refeeding and IP glucose injection, despite the interneuron response remaining intact. These changes in DG responsiveness to feeding corresponded to impaired performance during hippocampus-dependent learning and memory tasks. Fiber photometry also revealed impaired GC and interneuron responses during memory tasks in the HFD mice. Strikingly, one-night fast followed by refeed before memory encoding completely rescued memory performance in HFD mice. Overall, these data demonstrate

GC's and interneurons in the DG dynamically and robustly respond to feeding status and glucose levels. Importantly, even short-term dietary changes, such as high fat diet, or an acute fast/refeed, can have significant impacts on DG function and subsequently modulate memory processing.
Word Count: 269

Disclosures: T. Landry: None. **S. Tart:** None. **L. Zhang:** None. **J. Song:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 648.03

Topic: F.08. Food and Water Intake and Energy Balance

Support: CONACYT Grant

Title: Administration of a hyperglucid-hyperlipidic diet causes metabolic deterioration, neural damage, and loss of recognition memory in rats.

Authors: *E. FUENTES[1], A. D. DIAZ[2], M. A. J. GUEVARA[3], B. VENEGAS MENESES[4], G. FLORES[5], R. A. VAZQUEZ-ROQUE, Sr.[6], S. TREVINO MORA[7];
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Abstract: The number of people diagnosed with metabolic syndrome (MetS) has increased dramatically to reach alarming epidemic proportions worldwide. The origin of MetS derives from bad eating habits mainly deals rich in carbohydrates and saturated fat. In recent years, it has been reported that MetS can also promote the appearance of neurodegenerative diseases such as Alzheimer's dementia. Although the molecular mechanisms induced by MetS to cause neurodegeneration are not fully understood. We worked with Male Wistar rats of 30 days of age, they were randomly divided into four groups: Normocaloric diet (NCD), Hypercaloric-hyperglucid diet (HCD), Hypercaloric-hyperlipidic diet (HFD) and a mixture of HCD + HFD (50-50). The diets were administered for 90 days ad libitum. The weight, size, abdominal diameter, body mass index and fat percentage were recorded weekly. The Novel Objects Recognition Task was carried out on day 90 after the start of the administration of the diet to assess recognition memory. Blood samples were taken by the quantification of basal glucose and insulin, total lipids, triglycerides, free fatty acids, high, very low and low-density lipoproteins. Five animals from each group were decapitated, the brains were extracted and dissect the frontal cortex, hippocampus, and hypothalamus and were used to quantify reactive oxygen species, lipid peroxidation, Superoxide Dismutase and Catalase and pro-inflammatory cytokines. Seven brains for group were extracted and processed to immunohistochemical technique by identify COX-2

and GFAP. Six brains for group were dissected and stained with Golgi-Cox, the dendritic spines of neurons were drawn, and the number of spines was quantified. The results shown that the MetS induced by the consumption of hyperglycemic and hyperlipidic diets lead alterations at the central level, mediated by an exacerbated increase in oxidative stress and neuroinflammation at the level of the cortex, hippocampus, and hypothalamus. Events that together represent critical factors to trigger the deterioration in the structure and function of these brain regions. Therefore, the consumption of hyperglycemic and hyperlipidic diets causes a series of biochemical and metabolic alterations that impair recognition memory. Importantly, MetS poses a threat to quality of life for the global population because it could promote early onset of aging and consequently lead to the development of chronic degenerative diseases.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Topic: F.08. Food and Water Intake and Energy Balance

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Title: Continuous access to snacks in female rats from weaning onward causes fat accumulation in the liver before weight gain but does not cause hypothalamic inflammation

Authors: N. GENGLER, J. WONG, B. HAILE, J. DONAHUE, L. JACKSON, *H. I'ANSON; Biol., Washington & Lee Univ., Lexington, VA

Abstract: Obesity is characterized by chronic low-grade inflammation due to increased proinflammatory cytokine secretion and macrophage infiltration into metabolic tissues including the brain and liver. Microglia modulate brain inflammatory responses and their accumulation within discrete brain areas may vary with high-fat diets (HFDs). HFDs may also lead to lipid accumulation causing hepatic steatosis. Contrary to HFD studies, our snacking model closely mimics the trend towards increased snacking in humans. Our previous data showed that female rats eating snacks and chow from weaning had increased weight gain, abdominal fat pad weights, insulin insensitivity, and leptin resistance, all symptoms of metabolic syndrome (MS) (*Physiology & Behavior*, 201, 2019). In contrast to HFD-fed rats, there was no significant difference between snacking and control rats in arcuate nucleus POMC and astrocyte numbers or

hypothalamic POMC and NPY gene expression (*SfN* 2019, #681). Therefore, we investigated whether snacking causes low-grade inflammation centrally and/or peripherally by measuring (1) microglia accumulation in the hypothalamic arcuate nucleus and (2) lipid accumulation in the liver of our rat model. Female Long Evans rats were received at 21 days of age and provided continuous access to unhealthy snacks (Tostitos, Sandies, and peanut butter chips) and chow or only chow from Day 22 of age. Rats were terminated at 35, 56, 77, and 90 days of age (n=8 per group). Brain sections were processed for immunohistochemistry with Iba1 antibody (Fujifilm Wako Chemicals, 1:1000), and hypothalamic arcuate nucleus images were quantified for microglia (0.2mm x 0.2mm area, Adobe Photoshop). Liver sections were stained for lipid with Oil Red O and images of adjacent pairs of central veins and portal triads were taken in the same liver lobule for lipid quantification as percent lipid (lipid pixels per liver cell pixels in a 0.25mm x 0.25mm area, Adobe Photoshop). All results averaged two blind quantifiers. Our results showed no significant differences in microglia numbers between snacking and control groups nor were there any significant differences between rostral, medial, or caudal regions of the arcuate nucleus at any age. However, snacking rats had significantly more lipid accumulation than control rats in every age group ($n=8$, $p<0.05$). This study showed that snacking from weaning causes signs of MS, including liver hyperlipidemia, but no signs of hypothalamic inflammation at any time, from two weeks after snacking onset (Day 35 of age) to 68 days of snacking (Day 90 of age). These data suggest that snacking promotes peripheral changes in vulnerable tissues but not within the hypothalamus.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

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Program #/Poster #: 648.05

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant DK126740

Title: Convergent modulation of feeding and stress by an ascending catecholamine pathway

Authors: N. SAYAR-ATASOY[1], I. AKLAN[1], ***C. LAULE**[1], J. RYSTED[1], Y. YAVUZ[2], D. DAVIS[1], T. ATES[1], M. ONCUL[3], I. COBAN[4], H. KIM[1], B. YILMAZ[2], D. ATASOY[1]; [1]Neurosci. & Pharmacol., Univ. of Iowa, Iowa City, IA; [2]Yeditepe Univ., Istanbul, Turkey; [3]Biomed. Sci., Univ. of Leeds, Leeds, United Kingdom; [4]Anat. & Cell Biol., Ruprecht Karl Univ. of Heidelberg, Heidelberg, Germany

Abstract: Glucose is the main energy source for the brain and is under stringent regulation by the central nervous system. Potentially fatal decreases in blood sugar are countered by increased appetite (hypoglycemic hunger) which is impaired in diabetic patients who suffer from recurrent

bouts of hypoglycemia. Despite the danger this imposes, our understanding of the mechanisms that orchestrate hypoglycemic hunger are poorly understood. Earlier studies have shown that catecholaminergic projections to the paraventricular hypothalamus (PVH) are required for hypoglycemic hunger. The nucleus tractus solitarius (NTS) integrates ascending physiological information and broadcasts these messages throughout the brain to maintain homeostasis. Based on its established role in appetite, we hypothesized that catecholaminergic NTS neurons ($NTS^{DBH/TH}$) coordinate hypoglycemic hunger by inhibiting downstream satiety melanocortin-4 receptor expressing neurons in the PVH (PVH^{MC4R}). To test this, we employed a combination of *in vivo* and *ex vivo* cell-type specific circuit mapping techniques. We targeted axon-GCaMP6s to NTS^{DBH} and found that 2-deoxyglucose induced hypoglycemia robustly activates NTS^{DBH}→PVH projections. Consistently, imaging norepinephrine dynamics with the $GRAB_{NE}$ sensor revealed that hypoglycemia elicits norepinephrine release in the PVH. Functional assessment of this connection showed that optogenetic NTS^{TH}→PVH activation is orexigenic and chemogenetic PVH^{MC4R} activation abolished hypoglycemic feeding, illustrating a role for NTS^{DBH}→PVH^{MC4R} circuit in glucoprivic feeding. Moreover, we found that NTS^{DBH}→PVH^{MC4R} circuit is activated by other stressors such as LiCl, restraint, and tail suspension. Further analysis revealed that LiCl-induced conditioned place aversion requires PVH^{MC4R} silencing, highlighting a role for NTS^{DBH}→PVH^{MC4R} in stress response. Finally, we investigated signaling dynamics underpinning this connection. *In vivo* $NTS^{DBH:ChrimsonR}$→$PVH^{MC4R:GCaMP7s}$ circuit mapping, with bulk Ca^{2+} imaging, revealed mixed biphasic response with initial excitation followed by prolonged silencing. To elucidate connection dynamics at single-cell resolution, we performed *ex vivo* patch clamp with adrenergic antagonists. These experiments revealed that NTS^{DBH} activates and inhibits an equal proportion of PVH^{MC4R} (~25%) and these connections are mediated, in part, by alpha-adrenergic receptors. Taken together these studies show that adrenergic NTS^{DBH}→PVH^{MC4R} connections contributes to feeding and stress response.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Topic: F.08. Food and Water Intake and Energy Balance

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Title: Functional organization of GI information in the lateral parabrachial nucleus

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Abstract: Interoception, the sensing of signals from internal organs, is crucial to achieving homeostasis. For instance, appropriate food intake relies on accurate estimation of future energy balance, which in turn relies on gastrointestinal (GI) signals like nutrient content or stomach stretch. A key brain area that assesses visceral state is the lateral parabrachial nucleus (LPBN) in the brainstem. Visceral information is routed to the brain by vagal, spinal, and hormonal pathways, which converge in the LPBN to drive changes in behavior and physiology through forebrain and other projections. While some LPBN neurons are known to respond to GI signals and promote feeding cessation, we lack a comprehensive picture of the functional architecture and GI sensory preferences in the LPBN. We developed a novel method that enables recording of hundreds of LPBN neurons during chronic two-photon calcium imaging in both awake and anesthetized states. We used this approach to determine the functional organization of GI representations and their relation to natural feeding in LPBN. We first measured the visceral sensory preferences of LPBN neurons in response to mechanical stimulation across regions of the GI tract in anesthetized recordings. Our recordings show mixed selectivity for visceral organs: for example, some neurons respond only to stomach distention, some only to intestine stretch, and some to both. These data allow us to determine the spatial organization of these response patterns, including a putative viscerotopic map of internal organs. We also observe a range of response dynamics, where some neurons respond transiently to stretch, indicating they may be encoding changes in organ volume, while others show sustained activity, which may signal absolute volume. Our ongoing work involves relating these responses to awake recordings during ingestion, visceral malaise, and across fasted and fed states. Our preliminary data reveal a spatial wave of activation across neurons in a subregion of LPBN during food consumption, which we hypothesize can be mapped onto the GI sensory preferences of neurons measured during anesthetized recordings. These experiments lay the groundwork for understanding how interoceptive signals from the GI tract lead to satiety sensations, influence feeding behaviors, and ultimately regulate energy balance.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Program #/Poster #: 648.07

Topic: F.08. Food and Water Intake and Energy Balance

Title: Effect of intermittent fasting in hypothalamic-pituitary-thyroid axis function

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Abstract: Obesity is characterized by excessive lipid depots, it has become a pandemic and conventional diet-therapies, such as calorie restriction (CR), have failed. CR consists in reducing up to 50% of daily food intake, it reduces weight loss in rodents mainly due to muscle mass loss. This dietary regimen induces a negative energy balance (NEB) that downregulates the hypothalamic-pituitary-thyroid axis (HPT) function by decreasing thyrotropin-releasing hormone (TRH) expression in the hypothalamic paraventricular nucleus (PVN) despite the fall in serum thyroid hormones content. The inhibition of the HPT axis helps to preserve energy reserves, but it prevents weight loss in obese patients. An alternative dietary treatment for obesity is intermittent fasting (IF) characterized by alternate intervals of fasting and feeding. IF has different variants, such as time restricted feeding (TRF) in which the daily window for food consumption is restricted to 8-12 h, or the 5:2 fasting diet that restricts calorie intake to 30% of the requirements in 2 alternate days of the week. IF promotes weight loss without compromising muscle mass, improving insulin sensitivity, lipid oxidation and energy expenditure; however, HPT axis adaptation to body weight loss due to IF is unknown. Hence, we analyzed if the effects of intermittent fasting in body weight are mediated by an enhanced HPT axis function. Four groups of male rats were subjected to CR (50%), TRF (8 h feeding window), 5:2 fasting (70% restriction in fasting days) and control (C, *ad libitum* fed rats) for 4 weeks, their body weight and food intake were registered daily. After sacrificed we analyzed PVN TRH mRNA expression, T_3 and corticosterone serum contents. TRF rats decreased food intake vs C, but body weight was similar between IF (TRF and 5:2) and C groups. Despite that, mesenteric adipose tissue weight decreased in TRF and 5:2 groups in comparison to that of C rats. Regarding hormones serum content, corticosterone levels increased in CR, but values in TRF, 5:2, and C were similar. As expected, the CR-induced NEB decreased PVN TRH mRNA expression and T_3 serum content. In contrast, TRF and 5:2 groups showed similar PVN TRH mRNA expression and T_3 serum levels than C, maintaining the HPT axis function active and suggesting that energy expenditure is favored by IF regimens. In conclusion, the inhibition of HPT axis observed in CR can be prevented by intermittent fasting regimens, promoting enhanced energy expenditure and reducing adipose tissue. In addition, IF was not a stressful stimulus thus it could be a better dietary alternative than CR for the treatment of obesity.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

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Program #/Poster #: 648.08

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant R01 DK052849

Title: Serotonin acts on multiple receptors within the Nucleus of the Solitary Tract to differentially affect neuronal subpopulations.

Authors: *R. J. CALKINS[1], E. T. WINZENRIED[2], S. M. APPLEYARD[3];
[1]Integrative Physiol. and Neurosci., Washington State Univ. Grad. IPN, Pullman, WA; [2]Integrative Physiol. and Neurosci., Washington State Univ. IPN, Pullman, WA; [3]Washington State Univ., Washington State Univ., Pullman, WA

Abstract: The nucleus of the solitary tract (NTS) in the hindbrain is activated by the vagus nerve, including by gastrointestinal signals following a meal. NTS neurons integrate these signals with inputs from other brain regions before relaying their output throughout the brain to modulate feeding. NTS neurons are heterogeneous, with activation of most subpopulations resulting in decreased food intake. One neurotransmitter that inhibits food intake at the level of the NTS is serotonin (5HT). Our lab and others have shown that 5HT activates NTS neurons, including catecholamine and POMC neurons. 5HT drives the firing of catecholamine neurons through a presynaptic action at 5HT3 receptors to increase glutamate release from vagal afferents. 5HT depolarizes POMC neurons via a post-synaptic action at the 5HT2C receptor. However, the effects of 5HT on other NTS neuron subpopulations are less clear. Cholecystokinin (CCK) and leptin are critical satiety factors, and chemogenetic activation of NTS neurons that express either the leptin receptor (LepR) or cholecystokinin (CCK) inhibits food intake. Here we performed patch-clamp electrophysiology on semi-horizontal brain slices containing intact vagal afferent-to-NTS synapses from male and female mice aged 8-18 weeks. To identify LepR or CCK NTS neurons, mice expressing cre-recombinase under the control of the LepR or CCK promoters were crossed with Rosa-TdTomato floxed mice. In LepR neurons, bath application of 30 µM 5HT significantly increased the frequency of spontaneous excitatory post-synaptic current (sEPSC) in 5/25 and holding current in 13/25 neurons. It also significantly decreased sEPSC frequency in 4/25 and holding current in 5/25 neurons. In NTS CCK neurons, 5HT significantly increased sEPSC frequency in 7/14 and holding current in 5/14 neurons, as well as significantly decreased sEPSC frequency in 3/14 and holding current in 3/14 neurons. This suggests that 5HT has both presynaptic and post-synaptic effects in LepR and CCK NTS neurons. A 5HT3R agonist mimicked increased sEPSC frequency in CCK neurons, suggesting 5HT3Rs mediate presynaptic increases in glutamate release. Interestingly, 5HT had a much weaker effect to increase glutamate inputs onto LepR- and CCK-expressing NTS neurons than catecholamine neurons. The receptors mediating the post-synaptic effects of 5HT on LepR and CCK NTS neurons are being examined. These exploratory experiments indicate that 5HT alters the activity of different NTS neuronal subpopulations important for food intake through activation of multiple types of 5HT receptors. Given the importance of 5HT drugs for weight loss, understanding the actions of 5HT in the NTS is critical.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

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Topic: F.08. Food and Water Intake and Energy Balance

Support: NSF Grant IOS-1656626
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Title: Leptin administration into the raphe nuclei controls food intake through hypothalamic serotonin receptors

Authors: *N. D. MAXWELL[1], D. C. GILES[1], D. M. COLEMAN[1], A. T. SADEK[1], L. P. REAGAN[1,2], J. R. FADEL[1], C. A. GRILLO[1];
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Abstract: Leptin is an adipocyte-derived hormone that controls a myriad of homeostatic functions. Most notably, leptin acts on neurons in the hypothalamus through the long form of the leptin receptor to control energy homeostasis and appetite. Leptin has not only been shown to act on hypothalamic neurons, but also on other areas of the brain such as the dorsal raphe nucleus (DRN), the primary source of brain-derived serotonin (5-HT). Previously, we have described an anatomical connection between leptin sensitive neurons in the DRN and the arcuate nucleus of the hypothalamus in adult male Sprague-Dawley rats. Additionally, we have shown that 5-HT is primarily involved in leptin's ability to reduce food intake through the DRN. Although these connections have been described anatomically and have been shown to be important in the regulation of food intake, the mechanism of this signaling has yet to be fully explored. The 5-HT 2C receptor (5HT2CR) has been strongly implicated in the control of food intake and as has been a target for the treatment of obesity. However, the link between the 5HT2CR and leptin responsive serotonergic neurons in the DRN has yet to be elucidated. In the present study, our objectives were to 1) characterize the expression and distribution of the 5HT2CR subtype in the hypothalamic nuclei, and 2) investigate the functional importance of this receptor subtype in the reduction of food intake when leptin is infused into the DRN. To complete these objectives, we used RNAScope to label 5HT2CR mRNA expression across the hypothalamic nuclei, as well as RNAScope and immunohistochemistry to further characterize the neurons that express these receptors. To show the involvement of this receptor on feeding behavior, we infused the 5HT2CR antagonist SB242084 intracranially into the third ventricle followed by leptin into the DRN and measured food intake and locomotor activity. Compared to DRN-leptin treated animals, blocking hypothalamic 5HT2CRs prior to leptin administration to the DRN shows an increase in food intake, but does not completely block the anorectic effects of leptin. In addition to measuring food intake, we also determined the hypothalamic signaling pathway for 5HT2CR in presence or absence of the specific antagonist after leptin administration into the DRN. Through this study, we aim to further characterize the extra-hypothalamic pathways leptin utilizes to control food intake, especially through the interaction with the serotonin system.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 648.10

Topic: F.08. Food and Water Intake and Energy Balance

Support: Scientific and Engineering Research Board- Department of Science and Technology, Govt of India

Title: Allyl isothiocyanate protected against olanzapine-induced metabolic alterations in mice: involvement of hypothalamic energy sensing, appetite regulation and inflammatory aberrations

Authors: *R. K. SODHI[1], H. KUMAR[1], R. SINGH[2], Y. BANSAL[3], Y. SINGH[1], M. BISHNOI[4], A. KUHAD[1];
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Abstract: Olanzapine is one of the commonly prescribed atypical antipsychotics but has a higher propensity of inducing severe metabolic adverse effects. Allyl isothiocyanate (AITC) is an agonist of transient receptor potential ankyrin 1 (TRPA1) channels and has been reported to have antidiabetic and antiobesity potential in rodents as well as in humans. With this background, the present study was carried out to investigate the protective effects of AITC in olanzapine-induced metabolic alterations in mice. Female BALB/c mice were treated with olanzapine for six weeks, which showed increased feed intake, body weight and adiposity index, while reduced locomotion and body temperature. AITC treatment reversed the chronic olanzapine-induced metabolic alterations mainly increased serum glucose, lipids, insulin, proinflammatory cytokines, ghrelin, leptin and reduced adiponectin and liver glycogen levels. Olanzapine treatment also induced insulin resistance and glucose intolerance in the oral glucose tolerance test, which was reversed by AITC treatment. These peripheral changes were associated with altered mRNA expression of hypothalamic appetite-regulating (NPY, POMC, AgRP, CART, GSH-R1a, LEPR) and nutrient-sensing factors (ACC-1, AMPK, PI3K, Akt) as well as inflammatory genes. These hypothalamic alterations were reversed by AITC treatment. Olanzapine treatment also led to reduced TRPA1 mRNA expression in the hypothalamus indicating its downregulation and potential role in inducing metabolic alterations. Furthermore, these antidiabetic and antiobesity effects of AITC were abolished in the presence of TRPA1 antagonist HC-030031. Results of the present study suggested the protective effects of AITC against olanzapine-induced metabolic alterations. Thus, AITC supplementation could be used as an adjunct to improve the outcomes of antipsychotic therapy.

Disclosures: R.K. Sodhi: None. **H. Kumar:** None. **R. Singh:** None. **Y. Bansal:** None. **Y. Singh:** None. **M. Bishnoi:** None. **A. Kuhad:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 648.11

Topic: F.08. Food and Water Intake and Energy Balance

Support: JST SPRING, Grant Number JPMJSP2119
MEXT/JSPS KAKENHI Grant Number 19K10039

Title: Dexamethasone suppresses cisplatin-induced nausea in rats with area postrema lesions and bilateral subdiaphragmatic afferent vagotomy

Authors: *S. SU, E. KIKUCHI, T. YOSHIZAWA, T. INUI, M. FUNAHASHI;
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Abstract: Cisplatin is a well-known anti-cancer drug that induces serious side effects such as nausea and vomiting. We have already found cisplatin-induced conditioned taste aversion (CTA) in rats treated with both area postrema lesions (APX) and bilateral subdiaphragmatic afferent vagotomy (VX). This is inconsistent with earlier reports that cisplatin-induced vomiting in ferrets is greatly attenuated by vagotomy. Therefore, we investigated the details of the pathogenesis of cisplatin-induced nausea in rats using behavioral experiments analyzing CTA. We used male Sprague-Dawley rats (6-7 weeks old at the beginning of the experiment). Half of the rats received APX and VX surgery. All rats were acclimated to planned drinking with periods of water deprivation for a week. Then some rats were conditioned with 0.1% saccharin solution paired with cisplatin (3 mg/kg, 1%BW, i.p.) with pre-administration of dexamethasone (1mg/kg, 0.1%BW, i.p.) 30 minutes before the administration of cisplatin, and the other rats were conditioned in the same manner without pretreatment of dexamethasone. Some rats were conditioned with 0.1% saccharin solution paired with dexamethasone. We measured saccharin intake for 20 minutes on the conditioning day and each test day. In addition, we performed a taste reactivity test (TR test) to observe a gaping reaction induced by re-administration of 0.1% saccharin (0.5ml/min, 8 min, p.o.) in rats acquired cisplatin-induced CTA to saccharin.The intact rats showed a significant reduction of saccharin intake after conditioning with cisplatin (n = 6), and pretreatment of dexamethasone failed to block the CTA acquisition (n = 6). Pretreatment with dexamethasone significantly suppressed cisplatin-induced CTA in rats treated with VX and APX on the 1st and 2nd test days (n = 6). These results suggest that the nervous system other than the area postrema and the vagal afferent inputs is also involved in the induction mechanism of cisplatin-induced nausea, and dexamethasone may have acted on such site. Gaping responses were produced by re-administration of 0.1% saccharin in rats with cisplatin-induced CTA, indicating that conditioned nausea is involved in the mechanism of cisplatin-induced CTA.

Disclosures: S. Su: None. **E. Kikuchi:** None. **T. Yoshizawa:** None. **T. Inui:** None. **M. Funahashi:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Program #/Poster #: 648.12

Topic: F.08. Food and Water Intake and Energy Balance

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MOST Grant 110-2314-B-006-114
MOST Grant 110-2320-B-006-018-MY3

Title: Microbiota modulates locomotion via vagus-dependent GLP-1 signaling

Authors: *T.-T. LAI[1,2], C.-H. FAN[3], Y.-T. HOU[3], W.-L. WU[1,2,4];
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Abstract: Locomotor activity is a fundamental behavior that can be triggered by gut-driven motivation status to drive animals from one place to another. Gut-driven behavior is controlled by various gut neuropeptides that can act as hormones or signal through the vagus nerve. Recent studies suggest that the levels of gut hormones can be driven by the colonization status of gut microbes, suggesting complex interactions among gut bacteria, gut hormones, brain, and host behavior. Herein, depletion of the gut microbiome by broad-spectrum antibiotic cocktail (ABX) in mice led to decreased locomotion and elevated gut hormone glucagon-like peptide-1 (GLP- 1). Antagonism of GLP-1 receptors or subdiaphragmatic vagotomy (SDV) procedure successfully reversed the hypolocomotion in ABX mice. In addition, the neurons in the vagal ascending brain regions were activated in ABX mice after the locomotor activity test. The hypolocomotion can be recapitulated in mice with intact microbiome by chemogenetic activation and ultrasonic neuromodulation of neurons in vagal ascending brain regions. Finally, we identified key bacteria modulating GLP-1 by selective antibiotic treatment. Colonization of the specific bacteria in antibiotic-treated mice suppressed the GLP-1 levels and restored their locomotion. These findings reveal that the gut microbiome modulates locomotion through GLP-1 signaling in the vagal circuit.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Program #/Poster #: 648.13

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant DK107500
NIH Grant DK133818

Title: Convergent approaches to identify targets of glucagon-like peptide-1 in the control of fluid intake

Authors: *D. J. BRAKEY[1], G. VARAVENKATARAMAN[1], K. C. SCHATZ[2], M. J. PAUL[2], D. DANIELS[3];
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Abstract: Although much is known about the central structures that drive fluid intake, less is known about the mechanism of thirst satiation. There is evidence that glucagon-like peptide-1 (GLP-1) serves as a satiety signal for fluid intake, but the relevant sites of action are unknown. To better define the circuits controlling intake satiation, particularly via GLP-1, our lab employed two approaches. First, we used anterograde tracing to identify projections from the nucleus of the solitary tract (NTS; the primary source of central GLP-1) and tested for drinking-induced Fos in the identified targets. Male and female rats were given NTS injections of an AAV that is transported anterogradely. One month after virus injection, rats were either water-deprived or non-water-deprived for 24 hours and subsequently either allowed to drink or not for 2 hours before transcardial perfusion. Preliminary analyses of brains from male rats found several regions containing both AAV-labeled fibers and Fos. Some of these regions, such as the *organum vasculosum of the lamina terminalis*, the median preoptic nucleus, and the paraventricular hypothalamic nucleus, have been implicated in the control of fluid intake. Additionally, we found overlap between AAV and Fos in the paraventricular thalamic nucleus, an area far less understood with respect to fluid intake. Ongoing analysis is testing the hypothesis that activity within these NTS targets is induced by fluid intake, rather than by dehydration alone. Second, we used vasopressin-deficient Brattleboro rats, which have normal food intake, but copious water intake. Of particular importance in this respect is our previous finding that Brattleboro rats are hypersensitive to the suppressive effects of the GLP-1 receptor agonist (Ex4) on fluid intake, but not on food intake. Using this model, we evaluated the response to GLP-1 and to drinking, allowing for a comparison of these responses that will help draw conclusions about the role of GLP-1 in drinking. Analysis of brains from male and female wildtype and Brattleboro rats that were either given injections of Ex4 or dehydrated (with or without subsequent water intake) revealed genotype-dependent differences in the pattern of central activity in response to the treatments. In the NTS, supraoptic nucleus, and subfornical organ, there were drinking-induced differences in Fos observed in wildtype rats, but not Brattleboro rats. The response to Ex4 in the NTS was greater in Brattleboro rats than in wildtype rats. Ongoing analyses are examining additional brain regions. These experiments offer a powerful combination of approaches to identify targets of GLP-1 in the control of thirst satiety.

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Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Program #/Poster #: 648.14

Topic: F.08. Food and Water Intake and Energy Balance

Support: RF1MH120144

Title: Glp-1 signaling in the paraventricular-dorsal vagal complex circuit

Authors: *L. WANG[1], Y. LU[1], M. BERNABUCCI[2], Z. P. PANG[1];
[1]Neurosci., The Child Hlth. Inst. of NJ, NEW BRUNSWICK, NJ; [2]Utsouthwestern, Utsouthwestern, Dallas, TX

Abstract: Central nervous system (CNS) control of metabolism plays a pivotal role in maintaining energy homeostasis. Glucagon-like peptide 1 (GLP-1, encoded by *Gcg*), secreted by a distinct population of neurons located within the Nucleus Tractus solitarius, suppresses feeding. Central and peripheral GLP-1 work independently to suppress feeding. However, *the cellular and circuit mechanisms mediating endogenous GLP-1 action in the CNS are still poorly understood.* This is mainly due to the presence of diverse neuronal subtypes, complex central neuronal connectivity, and the lack of molecular tools that can directly detect GLP-1 release in the brain. Our overarching goal is to gain a mechanistic understanding of endogenous GLP-1 release and its functions in the CNS in a cell type- and circuit-defined manner. In a previous study, we found that NTS GLP-1 projection to the paraventricular hypothalamic nucleus (PVN) enhances glutamatergic synaptic transmission, which is sufficient to suppress food intake, and ablation of PVN GLP-1R causes overeating and obesity. One major projection of PVN GLP-1R-expressing neurons is to the brain stem DMV region. We then asked whether the PVN GLP-1R-expressing neurons form synapses on the lateral section of the DMV cholinergic neurons. Whole-cell patch clamp was done in neurons from the DMV. Using optogenetic stimulation of the PVN GLP-1R-axon terminals in the DMV, we can induce reliable EPSCs which could be blocked by CNQX. The application of GLP-1R specific agonist Exn-4 enhanced the frequency of the spontaneous EPSCs and augmented the PVN GLP-1R-neurons-to-DMV neuronal excitatory strength**.** Since DMV neuronal activity regulates glucose homeostasis, the PVN GLP-1R-neurons to DMV excitatory input suggests that this neurocircuit may play role in regulating energy metabolism. We next conducted loss-of-function studies by inactivating synaptic transmission in this pathway and investigated its impact. To block the PVN GLP1R-to-DMV synaptic release, we used targeted expression of tetanus toxin light chain (TeNT) to cleave SNARE proteins required for synaptic release in *GLP-1-ires-Cre.* Cre-dependent Retro-AAV-DIO-FLPo was injected into the DMV region, and pAAV-fDIO-TeNT was injected into the PVN. In this manner, the TeNT is specifically expressed in the PVN GLP-1R neurons that are projected to the DMV region to block the release of neurotransmitters. Compared to control animals, mice expressing TeNT developed clear metabolic issues, including increased body weight, elevated blood glucose, and deficits in glucose metabolism. These results highlight the potential role of central GLP-1 in regulating energy homeostasis.

Disclosures: **L. Wang:** None. **Y. Lu:** None. **M. Bernabucci:** None. **Z.P. Pang:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Program #/Poster #: 648.15

Topic: F.08. Food and Water Intake and Energy Balance

Support: AHA Postdoc Fellowship # 20POST35120600

Title: A food odor-responsive neuro-circuit regulates food intake

Authors: *Y. HE, H. LIU, Y. XU;
Baylor Col. of Med., Houston, TX

Abstract: Olfactory inputs play an important role in eating behavior, including triggering appetite, facilitating food seeking and influencing food choice. While transient food stimuli can influence appetite and trigger food cravings in the hungry state, long-term exposure to food-related odor induces satiation and therefore prevents overeating. However, the mechanisms by which olfaction regulates satiation and food intake remain unknown. Here, we show that prolonged food odor exposure suppresses both chow and high fat diet intake in mice. Further, we found that a subset of neurons in the ventral subiculum (vSub) are activated by food odor and that the ventromedial hypothalamus (VMH) is a critical downstream substrate for food odor-mediated hypophagia. Activation of VMH-projecting vSub neurons suppresses food intake and enhances olfactory sensation to food. Additional results show that the VMH-projecting vSub neurons receive the projections from the olfactory bulb. Together, these findings reveal a novel neurobiological circuitry mediating olfactory signaling that regulates food intake.

Disclosures: **Y. He:** None. **H. Liu:** None. **Y. Xu:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 648.16

Topic: F.08. Food and Water Intake and Energy Balance

Support: MOST 110-2326-B-038-002-MY3
CGMH Grant CMRPD1M0121

Title: Ablation of NPFFR2 in Mice Reduces Response to Type 2 Diabetes Model

Authors: *Y.-T. LIN[1], J.-C. CHEN[2];
[1]Grad. Inst. of Metabolism and Obesity Sci., Taipei Med. Univ., Taipei, Taiwan; [2]Grad. Inst. of Biomed. Sci., Chang Gung Univ., Tao-Yuan, Taiwan

Abstract: Neuropeptide FF (NPFF) belongs to a RF-NH2 peptide family and is recognized as a morphine modulating peptide. NPFF involves in different physiological functions, including the regulation of analgesic effect of opioids, and controls food consumption and cardiovascular function through its interaction with two cognate receptors, NPFFR1 and NPFFR2. NPFF is highly expressed in the hypothalamus, and has been reported to regulate feeding behavior and energy homeostasis. Our previous results demonstrate that NPFF inhibits the insulin-stimulated signaling pathway and appetite-relating peptide expression in mice hypothalamus. Insulin was known to modulate the peripheral metabolism through downstream signals sent from the central nervous system, including liver gluconeogenesis, lipid metabolism and adipose tissue thermogenesis. To investigate the impacts of NPFF on metabolic disorders, an HFD/STZ-induced type 2 diabetes model was adopted in WT and NPFFR2 knockout mice. In the current report, we found that the deletion of NPFFR2 eliminated the HFD/STZ-induced central insulin resistance. And NPFFR2 knockout ameliorated the metabolic phenotypes like the imbalance of glucose homeostasis and impaired lipid oxidation. Our evidence shows that the ablation of NPFFR2 attenuates the metabolic symptoms in the type 2 diabetes model.

Disclosures: Y. Lin: None. **J. Chen:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 648.17

Topic: F.06. Autonomic Regulation

Title: Role of insulin receptors expressed by the autonomic preganglionic neurons

Authors: *U. HYUN, J.-W. SOHN;
Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Insulin is a peptide hormone that regulates post-prandial physiology. It is well-known that insulin controls homeostasis via its receptors in the central nervous system (CNS), but the role of insulin receptors (InsRs) expressed by the autonomic nervous system (ANS) is currently unknown. In this study, we used the ChAT-IRES-Cre mouse model to label cholinergic preganglionic neurons of the ANS to study the role of InsRs expressed by the autonomic neurons. Specifically, we observed the acute inhibitory effects of insulin on autonomic neurons by patch-clamp electrophysiology experiments. We also inspected the autonomic function of InsRs expressed by the autonomic preganglionic neurons by crossing the $InsR^{flox/flox}$ mice with the ChAT-IRES-Cre mice. Our results would provide insights how insulin affects autonomic neurons to regulate homeostasis.

Disclosures: **U. Hyun:** None. **J. Sohn:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

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Program #/Poster #: 648.18

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH R35 ESI-MIRA Grant (R35GM133698-01)
Pew Biomedical Scholar Award
the Alfred P. Sloan Foundation Award

Title: In1 neurons regulate food ingestion through gut-brain axis in drosophila

Authors: *X. CUI, M. MEISELMAN, H. KIM, N. YAPICI;
Cornell Univ., Ithaca, NY

Abstract: Food intake is essential for all animals to survive. In many animals, food intake is strictly regulated by sensory, homeostatic, and hedonic neural circuits, which balance energy intake with energy expenditure. Although neural circuits that regulate food intake have been extensively investigated in rodent models, the entire sensory-motor neural circuits that generate food perception and accordingly regulate food intake behavior have not been fully understood in any model organism. We use the fruit fly (*Drosophila melanogaster*) to understand the fundamental principles of how the brain integrates the sensory perception of food with the sensation of hunger to regulate food intake on the neural circuitry level. Previously, we identified a novel group of interneurons, IN1 neurons, as a regulator of food ingestion in flies. Here, we used optogenetics and two-photon calcium imaging to investigate the neural circuitry of IN1 neurons. We revealed that IN1 neurons receive specific excitatory input from sugar sensing chemosensory neurons and mechanosensitive neurons that respond to food texture. Next, we tested which sugar sensing neurons activate IN1 neurons. Our detailed analysis showed that taste neurons expressing the Gr43a taste receptor generated a strong and sustained calcium response in IN1 neurons. We further investigated which Gr43a neurons produce this excitatory effect and found that IN1 neurons receive excitatory input from interoceptive Gr43a neurons that innervate the fly gut. We developed a new imaging preparation named *Drosophila* gut live imaging dissection (GLID) to capture the activity of gut neurons *in vivo* in behaving flies. Using this imaging prep, we showed that Gr43a gut neurons indeed respond to sugar ingestion. Our research revealed that the gut-brain axis might regulate food ingestion in flies in a similar way to in rodents and humans.

Disclosures: **X. Cui:** None. **M. Meiselman:** None. **H. Kim:** None. **N. Yapici:** None.

Poster

648. Integration of Peripheral Signals on Feeding and Drinking

Location: SDCC Halls B-H

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Program #/Poster #: 648.19

Topic: F.08. Food and Water Intake and Energy Balance

Support: MEXT/JSPS KAKENHI Grant Number 19K10039

Title: Gaping reactions induced by emetine

Authors: *M. FUNAHASHI, S. SU, E. KIKUCHI, Z. WEI, H. HUANG, T. YOSHIZAWA, T. INUI;
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Abstract: Emetine is an ingredient of the root of Cephaelis Ipecacuanha, and its oral administration cause acute severe vomiting. Although rats can not vomit, we previously reported emetine-induced conditioned taste avoidance (CTA) to saccharin in rats. So we suggested that emetine-induced conditioned nausea is involved in the acquisition process of CTA. To investigate more details of the emetogenic effects and conditioned mechanism of emetine, we investigated the gaping reactions using taste reactivity test (TR test) in rats. Male Splague-Dawley rats (6-7 weeks old at the beginning of the experiment) were used. Surgery was performed on rats to install an oral catheter under anesthesia with a combination anesthetic containing (mg/Kg, i.p.): 0.15 medetomidine, 2 midazolam and 2.5 butorphanol. To analyze gaping reactions, the mouth movements of rats were recorded on video. The gaping reaction can be identified by the action of opening the mouth wide, and we measured the number of times of gaping using offline video images. We performed TR test with administration of 0.1% saccharin (0.5ml/min, 8 min, p.o.) in rats that acquired CTA to saccharine by the conditioning with emetine (5.54 mg/kg, i.p., 1% BW), and another TR test with administration of 10 mM emetine (0.5ml/min, 8 min, p.o.) in intact rats. The number of gaping reactions in rats acquired emetine-induced CTA to saccharine was 58.0 ± 10.7 times / 8 minutes (n = 5). When emetine was administrated orally, the number of gaping reactions was 67 ± 11.4 times / 2 minutes (n = 4). These results indicate the conditioned nausea produced by emetine-induced CTA to saccharin, suggesting the aversive memory to sweetness produced by conditioning with emetine. These findings demonstrated that emetine induces conditioned nausea and that rats develop conditioned aversion to saccharin. It was also suggested that the emetic effect of emetine can be quantified by measuring gaping reactions even in rats that can not vomit.
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Poster

649. Dopamine, Behavior, and Neuronal Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 649.01

Topic: G.03. Motivation

Support: NIH Grant DA042895
NIMH T32-MH115886

Title: Dopamine encoding of valence and behavioral flexibility across striatal subregions

Authors: *K. N. BORNHOFT, T. O'NEAL, J. PROHOFSKY, A. WOLFF, B. T. SAUNDERS; Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Adaptive decision making relies on dynamic updating of learned associations, where environmental cues come to predict positive and negatively valenced stimuli, such as food or pain. A network of brain systems, including the striatum, are key for flexible cue-guided behaviors. Dopamine signaling in the striatum is critical for learning and maintenance of these Pavlovian conditioned behaviors. To understand the role of dopamine signaling across striatal subregions in dynamic valence discrimination, we developed a Pavlovian reversal task to model behavioral flexibility when cues predicting positive or negative outcomes suddenly switch valence. Rats were trained to associate tone A with a chocolate drink reward, tone B with foot-shock, and a third tone (CS-) with nothing. After 5 days of training, the outcomes associated with tones A and B were reversed and the new contingencies were trained for 5 more sessions. We then completed a second reversal, re-associating tones A and B with their original outcomes. Throughout these behavioral sessions, we recorded dopamine signaling in the nucleus accumbens (NAc) and dorsolateral striatum (DLS) using fiber photometry with the biosensor dLight. We found that rats reliably discriminated all three cues, and the valence-specific tones produced appropriate conditioned behaviors - the chocolate drink-predictive cue preferentially promoted conditioned port entries and the shock-predictive cue promoted conditioned freezing. At the first reversal, rat's behavior changed rapidly in response to the reversed reward-to-shock cue, but more slowly to the reversed shock-to-reward cue. Notably, at the second reversal, behavior in response to both cues reversed faster, within the first session. In the NAc we saw increased dopamine signaling to both reward delivery and reward cue presentations. The response to shock delivery and the shock cue varied across recordings, with some showing increased dopamine, and others decreased dopamine, to the negatively valenced stimuli. On the reversal sessions, these cue-evoked signals initially went away, then re-emerged in correlation with behavioral reversal. The pattern was somewhat different in the DLS, where reward delivery increased dopamine, but shock delivery reduced dopamine, across all recording sites. The chocolate drink predictive cues more reliably produced conditioned dopamine signals in the DLS that were reduced on the first session following reversal, compared to the shock cue. Together, these data suggest that dopamine signals in the striatum dynamically encode predictive cues of positive and negative valence, with subregional variation.

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Poster

649. Dopamine, Behavior, and Neuronal Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 649.02

Topic: G.03. Motivation

Support: NIH Grant DA042895
NIDA 5T32DA007234-35

Title: Pauses in VTA GABA neuron activity drive Pavlovian learning and behavioral reinforcement

Authors: *M. STELZNER, A. WOLFF, B. T. SAUNDERS;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Forming associations between environmental cues and biologically relevant outcomes is vital. These associations become foundational to decision making, driving both reward seeking and avoidance of aversive outcomes. The Ventral Tegmental Area (VTA), a major dopamine (DA) center in the brain, is essential to this process. A prominent example is the finding that optogenetic stimulation of DA neurons in the VTA can directly drive Pavlovian learning, turning a previously neutral sensory cue into a valuable conditioned stimulus. While the role of DA neurons in associative learning has been well demonstrated, there remains a relatively unexplored neuronal population in the VTA. One third of the neurons in the VTA are GABAergic, and while these neurons are known to contact local DA neurons and modulate their activity, their contribution to associative learning remains under explored. To investigate this, we virally targeted VTA GABA neurons in wildtype rats by delivering an adeno-associated virus with Cre expressed under the GAD1 promoter. We also infused a Cre-dependent inhibitory opsin or a Cre-dependent fluorescent Ca^{2+} reporter in separate groups of animals. In an optogenetic Pavlovian conditioning procedure, we then paired brief inhibition of VTA GABA neurons with a discrete audiovisual cue. We predicted that inhibiting VTA GABA cells would disinhibit DA neuron activity and drive associative learning, similar to direct stimulation of DA neurons. Our results suggest that inhibition of VTA GABA neurons produces conditioned behavior and psychomotor invigoration in a manner that is somewhat distinct from direct DA stimulation. We further found that GABA inhibition supports intracranial self-stimulation, a behavioral phenotype similar to that of DA stimulation. In ongoing experiments we are using fiber photometry to record GCaMP8f signals, a proxy for *in vivo* activity, in VTA GABA neurons during Pavlovian conditioning to further understand their contribution to these processes.

Disclosures: M. Stelzner: None. **A. Wolff:** None. **B.T. Saunders:** None.

Poster

649. Dopamine, Behavior, and Neuronal Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 649.03

Topic: G.03. Motivation

Support: R00 DA042895
T32 DA007234
F31 DA055482

Title: Superior colliculus projections to dopamine neurons alter movement during Pavlovian learning

Authors: *C. L. POISSON, C. HERUBIN, A. R. WOLFF, B. T. SAUNDERS;
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Abstract: Animals associate rewarding or threatening events with specific sensory stimuli (i.e. cues) through Pavlovian conditioning. It is well known that dopamine (DA) neurons of the ventral midbrain (ventral tegmental area- VTA, and substantia nigra pars compacta- SNc) are key to the formation and expression of cue-guided behaviors. However, it is unclear how these dopamine regions rapidly integrate sensory information about cues in order to create conditioned behavior. A candidate structure that processes sensory information and sends excitatory projections to DA neurons is the superior colliculus (SC). Here, we use optogenetics to manipulate SC projections to the VTA and SNc during a cue conditioning task. Unlike direct DA neuron stimulation, optogenetic activation of SC-DA projections does not drive learning to create conditioned behavior to an otherwise neutral cue. Instead, excitation of these projections produces unique motor output - changes in head, neck, and body orientation. Additionally, stimulation of SC-DA projections does not create real time place preference or support intracranial self stimulation, suggesting that the information signaled by this projection does not have a strong positive valence. Despite this, fiber photometry recordings demonstrate that optogenetic stimulation of SC terminals in the VTA/SNc increases dopamine neuron activity. These studies suggest that excitatory input to DA neurons from the SC has a subtle role in learning, including movement invigoration and postural control during reward-seeking behaviors. In ongoing studies we are recording deep layer SC neuron activity during a visual cue-sucrose Pavlovian conditioning task with fiber photometry. These results reveal SC dynamics that are time-locked to reward consumption and are modulated by the presence of predictive visual cues. These SC neurons are also sensitive to aversive events, such as shock. Overall, our work implicates that deep layer SC neurons are involved in Pavlovian conditioning, and that projections to dopamine regions, in particular, influence movements associated with cue-reward learning.

Disclosures: C.L. Poisson: None. **C. Herubin:** None. **A.R. Wolff:** None. **B.T. Saunders:** None.

Poster

649. Dopamine, Behavior, and Neuronal Circuits

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Title: Basolateral amygdala dopamine signals track emotionally salient events

Authors: *M. A. BRICKNER, A. R. WOLFF, L. ENGEL, M. J. THOMAS, B. T. SAUNDERS;
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Abstract: Brain circuits that control the discrimination between positive and negative events are essential for driving appropriate behavioral responses to rewards and threats. The basolateral amygdala (BLA) has canonically been considered the basis for conditioned fear memory; however, growing evidence indicates the BLA mediates encoding of valenced stimuli in general, beyond fear states. Notably, dopamine neurons in the ventral tegmental area (VTA) innervate the BLA, where D1 dopamine receptors (D1DR) are densely expressed. Dopamine axons in the BLA are activated by both positively and negatively valenced stimuli, but it is unknown how dopamine signaling in the BLA encodes valence-based learning. Here, we used fiber photometry to measure *in vivo* BLA dopamine dynamics in rats with dLight, a genetically encoded dopamine biosensor. BLA dopamine transmission was recorded (1) during unconditioned reward and footshock delivery, and (2) during learning in a Pavlovian discrimination task where the rats distinguish between four cues - a cue that predicts a threatening stimulus (a footshock), a cue that predicts a rewarding stimulus (sucrose), a neutral cue, and a safety cue, which signaled relief from shock. Phasic dopamine signals emerged in the BLA in response to both unconditioned sucrose consumption and unconditioned footshocks. Dopamine dynamics also appeared after valenced learning in response to predictive cues in the cue discrimination task. However, dopamine signals did not distinguish cue type, suggesting that dopamine transmission within the BLA may serve as a general saliency or state transition signal, rather than a value signal. Future research will include calcium imaging of the activity of projection-defined principal excitatory BLA neurons and functional manipulations of D1DR containing BLA neurons during valence discrimination learning.

Disclosures: M.A. Brickner: None. **A.R. Wolff:** None. **L. Engel:** None. **M.J. Thomas:** None. **B.T. Saunders:** None.

Poster

649. Dopamine, Behavior, and Neuronal Circuits

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Topic: G.03. Motivation

Support: NIH Grant DA042895
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Title: Vta dopamine neurons engage subregion-specific striatal dopamine signals during pavlovian learning

Authors: L. ENGEL[1], A. WOLFF[1], M. BLAKE[1], V. L. COLLINS[2], S. SINHA[3], *B. T. SAUNDERS[1];
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Abstract: Environmental cues, through Pavlovian learning, become conditioned stimuli that guide animals toward the acquisition of rewards (for example, food) by invigorating and directing seeking behavior. We have previously shown that brief optogenetic excitation of dopamine neurons, in temporal association with visual sensory cues, can instantiate those cues as conditioned stimuli that evoke conditioned movements. It remains unclear 1) how dopamine-neuron mediated, cue-evoked behavior is signaled by dopamine release downstream in striatal subregions and 2) how subregional signals evolve across stages of learning. Here, we made use of a genetically encoded dopamine biosensor (dLight) to monitor dopamine signaling in the nucleus accumbens core (NAC), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) with fiber photometry, while tracking detailed movement features during optogenetic Pavlovian cue conditioning of VTA dopamine neurons. Our results demonstrate a progressive recruitment of cue-evoked dopamine signaling across striatal subregions that correlates with different features of behavior. Cues paired with optogenetic activation of VTA dopamine neurons evoked dopamine release preferentially in the NAC early in training when behavioral responses were slower and directed toward the cue. Critically, these NAC signals got larger, rather than diminishing with extended training. As conditioning progressed, cue evoked signals also emerged in the DMS and DLS, when movement patterns became more vigorous and not directed at the cue. We found subregion specific heterogeneity in the relationship between cue-evoked signals and movement vigor. DMS cue-evoked signals predicted slower movements, while DLS signals predicted faster movements, and NAC signals were not correlated with movement. In additional studies, we found that brief optogenetic inhibition of DLS dopamine terminals at cue onset late, but not early, in training decreased the probability of cue-evoked locomotion and slowed the onset of movement initiation. Together our studies show dissociable, parallel functions for ventral and dorsal striatal dopamine signaling in guiding versus invigorating behaviors. Further, they suggest that large-scale plasticity across the striatal dopamine network emerges during Pavlovian learning to coordinate behavior.

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Poster

649. Dopamine, Behavior, and Neuronal Circuits

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Title: Dopamine D2 receptors in nucleus accumbens cholinergic interneurons increase impulsive choice

Authors: *J. CAVALLARO, J. YEISLEY, R. SETARA, J. FLOEDER, E. GALLO;
Biol. Sci., Fordham Univ., Bronx, NY

Abstract: Maladaptive impulsive choice, which is characterized by excessive preference for small, short-term rewards over larger, longer-term gains, is often observed in substance use disorders and other neuropsychiatric illnesses. The neural mechanisms underlying impulsive choice behavior are thought to involve alterations in dopamine neurotransmission in the nucleus accumbens (NAc), a brain region critical for reward. Cholinergic interneurons (CINs) of the NAc, which express dopamine D2 receptors (D2R), have emerged as key regulators of striatal output and local dopamine release. Despite these relevant functions, whether D2Rs expressed specifically in these neurons contribute to impulsive choice behavior is unknown.

To address this question, we first used Cre-dependent adeno-associated viruses to overexpress D2Rs or EGFP in NAc CINs of choline acetyltransferase (ChAT)-Cre mice (both sexes). Four weeks later, mice were trained on a delay discounting task which measures the choice between lever pressing for a small, immediate reward or for a large reward presented after increasing delays across sessions. In the absence of delays to either reward option, both groups showed a similar preference for the large reward choice, suggesting that D2R upregulation does not alter reward magnitude sensitivity. Both groups showed decreased choice of the large reward with increasing delays, but discounting was significantly steeper in D2R-overexpressing mice compared to controls (virus x delay: $F_{(5, 70)} = 6.13$, $p < 0.0001$, n = 8 mice/group), suggesting an increase in impulsive choice following D2R upregulation. We then tested the effect of selective deletion of the D2R gene in CINs using ChAT-IRES-Cre mice x $D2^{flx/flx}$ mice (CIN D2-KO). Compared to $D2^{flx/flx}$ controls, CIN D2-KO mice showed greater choice for the large reward option at longer delays, reflecting reduced impulsive choice.

To determine whether CIN D2Rs contribute to impulsive choice involving uncertainty costs, we tested the effects of CIN D2R overexpression and deletion in a probabilistic discounting task. Here, mice chose between a small reward delivered with 100% certainty and a large reward presented with decreasing probability (100% to 20%). In contrast to delay discounting, we found no significant effect of D2R upregulation or deletion in probabilistic discounting. Together, these results suggest that CIN D2Rs promote impulsive choice behavior involving temporal but not probability costs. Our findings show that D2R expression levels in CINs play a key role in delay-based impulsive choice, providing new insight into the mechanisms by which NAc dopamine regulates CIN function and impulsive behavior.

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Poster

649. Dopamine, Behavior, and Neuronal Circuits

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Title: Allopregnanolone regulation of spontaneous dopamine transient frequency and amplitude in freely-moving male and female rats

Authors: *M. H. MCFARLAND, A. G. EDMONDS, A. MORROW, D. L. ROBINSON; Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Neurosteroids are compounds that are synthesized *de novo* in the brain and influence neuronal activity. Allopregnanolone (ALLO), a neurosteroid that is a potent, positive allosteric modulator of gamma-aminobutyric acid type A (GABA-A) receptors has emerged as a drug with considerable potential in the treatment of mental and affective disorders, including substance use disorders and premenstrual dysphoric disorder. Moreover, ALLO is considered to have a better safety profile than other drugs that target GABA-A receptors, such as benzodiazepines. Previous work in our lab has shown that ALLO dose-dependently reduces electrically-evoked dopamine release in the nucleus accumbens (NAc) in anesthetized male and female rats, with female rats being less sensitive to ALLO than males during the proestrus stage of the cycle. However, it is possible that the dopamine measurements were impacted by anesthesia effects on GABAergic neurotransmission in addition to ALLO. Thus, the present study tested the hypothesis that systemic administration of ALLO in awake rats will dose-dependently decrease the amplitude of spontaneous dopamine transients, while concurrently increasing their frequency. To test this hypothesis, we used *in vivo* fast scan cyclic voltammetry. We measured spontaneous, phasic dopamine transients in the NAc of freely-moving male and female rats before and after systemic administration of 0.0 (vehicle), 7.5, and 15mg/kg ALLO. We systematically presented unexpected, novel stimuli to increase the probability of dopamine release. Preliminary data show that ALLO appears to reduce the frequency of dopamine transients in male and female rats, but does not alter dopamine transient amplitude. In males, 7.5mg/kg ALLO reduced the frequency of transients in 3/5 rats and 15mg/kg reduced the frequency of transients in 2/3 rats, while vehicle did not decrease transients (3 rats). In females, 15mg/kg ALLO reduced transients in 3/3 rats, while vehicle did not decrease transients in 2/3 rats. More experiments are being conducted to clarify the effect of ALLO on spontaneous transients. As release of dopamine transients in the NAc is often associated with motivational events, a lack of ALLO effect suggests that motivation and reward processes remain intact at these doses. While data collection is still in progress, the

results from this study will clarify the regulation of dopamine neurotransmission by ALLO, which has clinical implications for the use of ALLO as an alternative therapeutic to benzodiazepines to treat various psychiatric disorders.

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Poster

649. Dopamine, Behavior, and Neuronal Circuits

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Topic: G.03. Motivation

Support: NSF GRFP

Title: Overlapping VTA dopamine neuron responses to palatable reward and conspecifics

Authors: *A. MINERVA[1], L. WILLMORE[2], B. ENGELHARD[4], M. MURUGAN[5], B. MCMANNON[3], C. J. PENA[6], I. B. WITTEN[1];
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Abstract: Dopamine (DA) neurons of the ventral tegmental area (VTA) respond to food rewards and social stimuli, and are critical for both food and social motivation; however, it is unknown if the same or different DA neurons encode these different types of stimuli. To address this question, we performed 2-photon calcium imaging of VTA DA neurons in mice presented with food and social stimuli using a novel head-fixed stimulus delivery apparatus. There was significant overlap in the neurons responding to presentation of food and social stimuli. To assess how changes in internal state affected this overlap, we imaged the same neurons across days while varying the animals' hunger state and social experience. Hunger not only increased the number of food responsive neurons, but also further increased the overlap in social and food responsive neurons. Similarly, opposite-sex experience not only increased the number of socially responsive neurons, but also further increased the overlap in the number of food and social responsive neurons. These results suggest that VTA DA population responses shift to match changing internal states, in a manner consistent with overlapping representations of food and social stimuli. A possible mechanism for this overlap may be shared expression of feeding- and social-related genes across VTA DA neurons. To explore this hypothesis, we conducted single-nucleus RNA sequencing (snRNA-seq) of mouse VTA and examined the landscape of expression of feeding- and social-related neuropeptide receptor and conversion enzyme genes in DA neurons. Indeed, rather than distinct DA populations expressing genes for only feeding- or social-related neuropeptides, we found expression of these genes across DA neuron subclusters. Taken together, the overlap between in vivo food and social representations as well as feeding-

and social-related neuropeptide receptor gene expression in VTA DA neurons suggests the same DA population underlies these different behavioral motivations.

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Poster

649. Dopamine, Behavior, and Neuronal Circuits

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Topic: G.03. Motivation

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Title: Phenotyping medial preoptic efferents to the ventral tegmental area in male and female rats: Implications for sex differences in mesolimbic reward processing

Authors: *J. MARTZ[1], A. VASQUEZ[2], J. M. DOMINGUEZ[3];
[1]The Univ. of Texas at Austin, Austin, TX; [2]Psychology, Univ. of Texas At Austin, Austin, TX; [3]Dept Psychology, Univ. of Texas at Austin, Austin, TX

Abstract: The medial preoptic area (mPOA) plays an important role in the regulation of motivated behaviors. While its role in sexually dimorphic and naturally rewarding behaviors, such as parental care and sexual behavior, is well established, its role in sex differences in drug response is just beginning to be explored. The mPOA is a major site of neuroendocrine and sensory integration that influences the mesolimbic dopamine system through efferent connections with the VTA. Prior research indicates that these connections are primarily GABAergic and sensitive to sex-steroid hormones. However, whether there are sex differences in the extent and profile of these efferents remains unclear. To investigate this, male and female rats received iontophoretic injections of the retrograde tract-tracer fluorogold (FLG) into the VTA. Immunohistochemical staining was used to visualize and quantify co-labeled FLG-positive neurons with GABA, estrogen receptor alpha (ERα), and androgen receptor (AR). Results revealed a pattern of VTA innervation that was comparable between males and females, with more efferents emerging from the rostrocentral portion of the mPOA than the caudal portion. Additional results indicated that both males and females have the same percentage of GABAergic mPOA-VTA projections. However, differences emerged when investigating the hormonal profile of projections to the VTA, where females had a greater percentage of mPOA-VTA efferents that were sensitive to estrogen signaling and males had a greater percentage of mPOA-VTA efferents that were sensitive to androgen signaling. Lastly, we co-localized FLG-positive cells with both GABA and ERα to find that, while around 50% of GABA neurons expressed ERα in both males and females, very few of these co-localized cells projected to the VTA. Overall, our results indicate that sex differences are present in the sex-steroid hormone

content of mPOA-VTA efferents. Here, we discuss potential contributions of this pathway to sex differences in mesolimbic reward processing.

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Poster

649. Dopamine, Behavior, and Neuronal Circuits

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Title: Nucleus accumbens dopamine release encodes sunk cost

Authors: *G. TOUPONSE[1], A. R. WANG[1], B. S. BENTZLEY[1], R. C. MALENKA[2], N. ESHEL[2];
[2]Psychiatry and Behavioral Sci., [1]Stanford Univ., Stanford, CA

Abstract: Decision-making requires a consideration of both costs and benefits. Although mesolimbic dopamine (DA) plays an established role in reward-related decisions, there has been longstanding controversy over its sensitivity to costs vs benefits. Here we combined fiber photometry, optogenetics, and a task inspired by behavioral economics to independently vary costs and benefits and explore DA's role in both. Over five days of training, mice (n=12) learned to nosepoke for access to sucrose reward, increasing the number of rewards they earned per session ($p<0.001$) and decreasing their latency to consume each reward ($p<0.01$). After training, mice performed economic demand sessions, in which the number of pokes required per reward was varied every 10 minutes for 50 minutes. Within each session, reward was fixed at a single combination of sucrose concentration and quantity, but these values varied between sessions. We found that mouse behavior was sensitive to both cost and benefit: increasing the cost (i.e., number of pokes) reduced the number of rewards ($p<0.001$), while increasing the sucrose concentration ($p<0.001$) or the size of each reward ($p<0.001$) increased the amount of sucrose consumed. While mice performed the task, we used fiber photometry to measure DA release in the nucleus accumbens core (NAc) with the fluorescent sensor GRAB-DA. As expected, increasing the sucrose concentration ($p<0.05$) or quantity ($p<0.01$) increased DA release to the cue signaling reward availability. Surprisingly, increased cost also enhanced cued DA release ($p<0.01$), implying that NAc DA release may reflect sunk cost in addition to benefit. We

repeated this experiment in another cohort of mice (n=9) that expressed both GRAB-DA in the NAc and the excitatory opsin ChRMINE in VTA DA neurons, and compared their performance to a control cohort (n=5) expressing GRAB-DA in the NAc and the inert fluorophore mScarlet in VTA DA neurons. The task was identical except that instead of sucrose, each reward involved brief optogenetic stimulation of DA inputs in the NAc. We found that over training, ChRMINE mice learned to earn more rewards ($p<0.001$) and nosepoke more accurately ($p<0.01$), unlike the mScarlet control mice, which earned fewer rewards ($p<0.05$) and became less accurate ($p<0.05$). Surprisingly, similar to the sucrose experiment, optogenetically-induced DA release was greater after a greater number of nosepokes ($p<0.001$), even though the stimulation parameters were identical. Thus, NAc DA release - either in response to natural rewards or optogenetic stimulation - is exquisitely tuned to sunk cost. The mechanisms and downstream consequences of this tuning are under active investigation.

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Poster

650. Social Circuits

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Title: The circuit basis of social valence

Authors: *P. ESPINOSA, M. LUCCHINI, B. GIRARD, F. CAMPANELLI, V. TIRITICCO, C. BELLONE;
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Abstract: To decide whether to approach or avoid a conspecific, individuals need first to recognize the possibly positive (appetitive) or negative (aversive) valence of the stimulus and learn this association. The Nucleus Accumbens (NAc) is a key brain region of the mesocorticolimbic circuits for evaluating valence. However, how valence is codified at the synaptic level in a social context is still an open question.
Within the NAc, D1 receptor-containing Medium Spiny Neurons (MSNs) have been related to rewarding and motivational aspects of social behavior. Using a free social interaction paradigm and calcium imaging techniques, we demonstrated that D1-MSNs respond to positive and negative social valence stimuli. Using anatomical tracing and in vitro electrophysiological recordings, we found D1-MSN strongly connected with glutamatergic neurons from the Anterior

Insular Cortex (AIC) that also express D1Rs revealing a novel D1R-D1R top-down circuit between AIC and NAc. A day after social interaction, we evaluated glutamatergic synaptic parameters and found a dichotomous, long-term valence-dependent synaptic plasticity that occurs specifically in these neurons. Interestingly, these forms of plasticity are triggered by different firing frequencies from AIC inputs. Specifically, we showed that low-frequency stimulation drives positive valence-like plasticity, whereas high-frequency stimulation induces negative valence-like plasticity. By recruiting the same D1R positive neurons within the AIC-NAc circuit, we demonstrated long-term synaptic plasticity signatures of social valence tuned by firing frequency.

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Poster

650. Social Circuits

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Title: Cortical representations of conspecific sex shape social behavior

Authors: *L. KINGSBURY[1], S. HUANG[1], T. RAAM[1], L. S. YE[1], D. WEI[1], R. HU[1], L. YE[2], W. HONG[1];
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Abstract: A central question related to many social decisions is how animals integrate sex-specific cues from con- specifics and use this information to guide behavior. Using microendoscopic calcium imaging in mice, we find that sex information is represented in the dorsal medial prefrontal cortex (dmPFC) across excitatory and inhibitory neurons. These cells form a distributed code that differentiates the sex of conspecifics and is strengthened on the order of minutes with social experience. While males and females both represent sex identity in dmPFC neuron populations, male mice show stronger encoding of female cues, and the relative strength of these sex representations predicts sex preference behavior. Using activity-dependent optogenetic manipulations of natively active ensembles, we further show that these specific cortical representations modulate preference behavior in male animals toward interaction with

male and female conspecifics. Together, these results define a functional role for native representations of sex in shaping social behavior and reveal a neural mechanism in the cortex underlying male- versus female-directed sociality.

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Poster

650. Social Circuits

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KAKENHI (JP19H05467)

Title: A cortico-subcortical circuit plays a causal role in social reward monitoring and valuation

Authors: *A. NORITAKE[1], T. NINOMIYA[1], K. KOBAYASHI[2], M. ISODA[1];
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Abstract: Reward to others affects one's own motivation. Previous studies in the macaque suggest that this social, motivational process is mediated by the circuit from the medial prefrontal cortex (mPFC) to the lateral hypothalamus (LH) (Noritake et al., 2018, 2020). To test this possibility causally, we selectively blocked activities of mPFC neurons projecting to the LH by using a DREADD-based double viral vector infection technique. In our behavioral procedure, two monkeys facing each other were conditioned with visual stimuli predicting the reward probabilities for the self (a monkey undergoing intervention, M1) and its partner (M2). When the circuit from the MPFC to the LH was intact, M1's anticipatory licking was increased with increasing M1-reward probabilities, and it was decreased with increasing M2-reward probabilities. When the circuit was blocked, the effect on M1's anticipatory licking of the M2-reward probability, but not of the M1-reward probability, was significantly decreased. In parallel with this behavioral change, neural synchronization between the mPFC and LH, as indexed by field-field coherence, was decreased especially in the theta and alpha bands. These results indicate that the circuit from the mPFC to the LH plays a causal role in subjective reward valuation by taking other-reward information into account.

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Poster

650. Social Circuits

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Title: Corticotropin-releasing hormone signaling from prefrontal cortex to lateral septum supports social novelty preference

Authors: N. DE LEÓN REYES[1], P. SIERRA DIAZ[1], R. NOGUEIRA[2], A. RUIZ-PINO[1], Y. NOMURA[1], C. DE SOLIS[3], J. SHULKIN[4], A. ASOK[3], ***F. LEROY**[1];
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Abstract: Social preference, the decision to interact with one member of the same species over another, is a key feature of optimizing social interactions. In rodents, social preference relies on both extrinsic factors, such as sex, strain and kinship, and intrinsic ones, such as the memory of previous encounters, which favors interactions with novel compared to familiar animals (social novelty preference). At present, it is unclear which neuronal circuits guide social preferences and whether such circuits promote social interactions with the preferred individuals or suppress interactions with the non-preferred ones. Although both the infralimbic area of the prefrontal cortex (ILA) and the lateral septum (LS) have been shown to support social novelty preference, the neuronal circuits and molecular mechanisms by which these brain regions interact to regulate social interactions are unknown. Here, we identify a population of inhibitory neurons in ILA that express the neuropeptide corticotropin-releasing hormone (CRH) and project to the rostro-dorsal region of LS (rdLS). Release of CRH from ILA in rdLS during interactions with familiar mice disinhibits rdLS neurons, thereby suppressing interactions with familiar mice and contributing to social novelty preference. We further demonstrate how the maturation of CRH expression during the first two post-natal weeks enables the developmental shift from a preference for littermates in juveniles to a preference for novel mice in adults.

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Poster

650. Social Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 650.05

Topic: G.03. Motivation

Support: Fondation HUG ME12272
Swiss National Science Foundation
NCCR-Synapsy

Title: Self-supervised transformer predicts social behavior in mice

Authors: *G. CHINDEMI, B. GIRARD, C. BELLONE;
Univ. de Geneve, Geneva, Switzerland

Abstract: While naturalistic social behavior spans a rich and dynamic repertoire, animal research in Neuroscience is mostly restricted to a small subset of easily quantifiable actions, such as attacking or sniffing. In recent years, the popularization of deep learning methods for body pose estimation has drastically simplified the analysis of behavioral video recordings. However, the identification of behaviors of interest remains a subjective choice of the experimenter.
In this work we explore the use of transformers, a popular family of machine learning architectures, to discover and quantify social behavior from body pose estimates, without human intervention. We trained a custom transformer network on two self-supervised learning tasks to build an internal representation of social interactions in free behaving mice. We then identified recurrent patterns in the embedding space and found that they correspond to salient moments in the video recordings, matching with common social interactions annotated by humans. Finally, we used the method proposed here to characterize social behavior in a model of autism spectrum disorder.
Our model can contribute to stratify social behavioral ethograms, which are essential not only to advance our basic understanding of the functioning of the nervous system, but also to evaluate and categorize animal models related to psychiatric disorders and the development of brain-machine interfaces.

Disclosures: G. Chindemi: None. **B. Girard:** None. **C. Bellone:** None.

Poster

650. Social Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 650.06

Topic: G.03. Motivation

Support: NIH Grant MH119422

Title: The insular cortex and basolateral amygdala are necessary for social affective preference in female rats

Authors: *A. DJERDJAJ[1], N. S. RIEGER[2], B. N. CAREY[3], B. H. BRADY[3], A. J. NG[4], J. P. CHRISTIANSON[5];
[1]Boston Col., Cambridge, MA; [2]Psychology, [4]Psychology & Neurosci., [3]Boston Col., Chestnut Hill, MA; [5]Psychology & Neurosci., Univ. of Colorado, Chestnut Hill, MA

Abstract: Abnormal social behavior and corresponding brain network connectivity occurs in several psychiatric disorders, including schizophrenia and autism. Sex differences in the prevelance of these disorders warrants research into the corresponding neurobiology of socioemotional behaviors. The basolateral amygdala (BLA) and the insular cortex (IC) are reciprocally connected regions involved in social cognition and the majority of prior work has focused on their contributions to social behavior in male rats. Here, we investigated the functional role of these regions in female rats in a social affective preference (SAP) test in which experimental rats exhibit approach to stressed juvenile but avoidance of stressed adult conspecifics. In separate experiments, the IC and the BLA were inhibited by local infusion of muscimol (100ng/side in 0.5uL saline) or vehicle prior to SAP testing with either juvenile or adult conspecifics. In both cases, muscimol interfered with preference for the stressed juvenile and naive adult, indicating that these regions are necessary for appropriate social affective behavior. In male rats, social preference is mediated in part by modulation of insula excitability by oxytocin, but there are noteworthy sex differences in the oxytocin receptor distribution in rats. After the SAP test, oxytocin (OT, 500uM) was infused into the IC or BLA 15 minutes prior to social interaction tests with naive juveniles or adults. OT infusion into the BLA, but not the IC, increased social investigation. To summarize, both male and female rats recruit the BLA and IC in social affective preference tests but OT may contribute to these circuits in sex specific ways--augmenting insular excitabilty in males and modulating BLA activity in females. Ongoing work seeks to characterize the effect of OT on female BLA function using in vitro electrophysiological methods.

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Poster

650. Social Circuits

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 650.07

Topic: G.03. Motivation

Support: NIH K01 MH119540

Title: Basolateral amygdala activity during social approach and interaction

Authors: *S. L. FERRI[1], S. A. HEINEY[2], P. QUINONES[3], T. ABEL[3];
[1]Univ. of Iowa, IOWA CITY, IA; [3]Dept. of Neurosci. and Pharmacol., [2]Univ. of Iowa, Iowa City, IA

Abstract: Social behaviors are present in different forms in nearly all species and are vital to survival, from transmitting cues of danger to promoting reproduction. These behaviors are highly complex but the neuronal population and circuitry underlying them are not thoroughly defined. Because of their importance in daily life for most organisms and their vulnerability to disruption in neuropsychiatric disorders such as schizophrenia and Autism Spectrum Disorder, it is crucial to define the circuit and molecular signatures of social behavior. While many brain areas, cell types, and genes are involved in social behaviors, we are focusing on neuronal activity in the basolateral amygdala (BLA) during social affiliative behaviors in which sexual and aggressive motivations have been minimized. Our preliminary data using a genetically encoded calcium indicator and fiber photometry system during the three-chamber social approach test and free social interaction indicate that glutamatergic BLA neurons are significantly more active during social sniffing than during investigation of a novel object or when the mouse is at rest, in a time-locked manner, as measured by %ΔF/F. In addition, we find specific patterns of BLA activation during discrete events of free social interaction. Future experiments will target specific BLA neuronal populations and further parse out their role in discrete social interaction behaviors.

Disclosures: S.L. Ferri: None. **S.A. Heiney:** None. **P. Quinones:** None. **T. Abel:** None.

Poster

650. Social Circuits

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Program #/Poster #: 650.08

Topic: G.03. Motivation

Support: JSPS KAKENHI Grant Number 19K21806
JSPS KAKENHI Grant Number 19J20173
Sasakawa Scientific Research Grant Number 2022-6032

Title: Biobehavioral Characteristics of Adult Rats that Experienced Brief Social Isolation

Authors: *M. TOYOSHIMA[1,2], K. YAMADA[1];
[1]Univ. of Tsukuba, Tsukuba, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan

Abstract: “Loneliness” is a common social problem associated with severe physical and mental diseases, including cardiac disorders, Alzheimer’s disease, and depression. In aging populations, older adults frequently feel lonely due to the death of intimate persons. In addition, the Covid-19 pandemic still has restricted people from face-to-face communication, making them feel lonely. Although its severity, pharmacological treatments and clinical interventions for loneliness have not yet been developed, mainly due to a lack of appropriate animal models. Therefore, biobehavioral characteristics of a loneliness-like state and its neural mechanisms in rodents are emergency issues. Recently, it has been reported that brief social isolation/separation facilitates social contact behaviors caused by negative emotional states that may reflect a loneliness-like state in adult rats and mice (Matthews et al., 2016; Ferrara et al., 2021; Fukumitsu et al., 2022).

However, further investigation of biobehavioral aspects of loneliness is needed to achieve a comprehensive understanding of loneliness. In the present study, we evaluate behavioral characteristics in briefly isolated adult rats. First, we assessed the effects of brief isolation on social approach behaviors toward various social stimuli. Group-housed rats showed increased approach behaviors toward novel, but not their cage mate, conspecifics, while rats that were isolated for a day exhibited higher social motivation independent of the familiarity of stimuli. Furthermore, the brief isolated rats more approached empty cylinders that conspecifics had previously existed than the group-housed ones. These results suggest that experience-dependent self-states, regardless of stimulus types, contribute to increased social approach behaviors in the isolated rats. Since lonely people tend to be vulnerable to anxiety (Cacioppo et al., 2006), we next focused on an association between isolation-induced social motivation and anxiety in rats. Anxiety levels in an elevated plus-maze test were positively correlated with social approach behaviors in the isolated, but not group-housed, rats. The fact that the anxiety levels in the isolated rats did not correlate with social motivation before the brief isolation assumes that the experience of isolation, but not inherent individual differences in emotional/ motivational sates, may cause the quantitative association between anxiety and motivation levels. In addition, we are investigating neural activities during social approach behaviors using c-Fos immunohistochemistry to evaluate neuroanatomical characteristics of the briefly isolated rats.

Disclosures: **M. Toyoshima:** None. **K. Yamada:** None.

Poster

650. Social Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 650.09

Title: WITHDRAWN

Poster

650. Social Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 650.10

Title: WITHDRAWN

Poster

650. Social Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 650.11

Topic: H.08. Learning and Memory

Support: NSERC

Title: The role of dihydrotestosterone in dorsal hippocampal D2-type dopamine receptor regulated social learning in male mice

Authors: *N. BASS[1], M. LECLAIR[1], E. CHOLERIS[2];
[2]Psychology, [1]Univ. of Guelph, Guelph, ON, Canada

Abstract: Social learning, a critical, common, and evolutionarily adaptive form of learning, may be defined as "learning that occurs via the observation of, or interaction with, a conspecific or it's products" (Heyes, 1994; Galef, 1998). The neurobiological mechanisms underlying social learning are poorly understood, but in animals may be studied using the social transmission of food preference (STFP) paradigm. By utilizing the STFP, the dopaminergic system, the dorsal hippocampus (DH), estrogens, progesterone and androgens have been implicated in social learning. Our previous work revealed that DH D2-type dopamine (DA) receptor antagonism blocked social learning in castrated male mice, but not gonadally intact males. The gonads produce that majority of the circulating sex hormones, so we followed up by castrating mice and replacing specific hormones one at a time to determine which are interacting with D2-receptors in the DH to regulate social learning. In the male brain, gonadal hormones may act either directly at androgen receptors, or indirectly at estrogen receptors following aromatization. Our preliminary findings revealed that long-term estradiol and progesterone treatment (in separate studies) protected against the impairing effects of DH D2-type DA receptor antagonism on social learning in castrated male mice. The purpose of this study was to elucidate whether DH D2-type DA receptors interplay with androgens to regulate social learning in castrated male mice. To test this, adult castrated male "observers" (OBS) are implanted with long-term subcutaneous slow releasing dihydrotestosterone (DHT), a potent androgen, or vehicle silastic capsules. OBS then received acute bilateral infusions of the D2-type DA receptor antagonist raclopride (20 µg/µL) into the DH 10-minutes before a 30-minute social interaction with a recently fed, same-sex, familiar "demonstrator" (DEM). Immediately following the social interaction, OBSs undergo an 8-hour choice test where they have free access to two novel flavored food diets. If social learning occurs, the OBS prefers the DEM diet. Because our preliminary findings showed that estrogens and progesterone protected against the impairing effects of intra-DH D2-type DA receptor antagonism on social learning in castrated males, perhaps all hormones can influence the effects of DH D2-type DA receptor antagonism on social learning. Thus, it is predicted that we will see similar results in the present study. Funded by NSERC.

Disclosures: N. Bass: None. **M. Leclair:** None. **E. Choleris:** None.

Poster

650. Social Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 650.12

Topic: G.03. Motivation

Support: ERC Grant 864552
SNSF Grant 31003A_182326

Title: Role of VTA-dopaminergic neurons in the social motivation deficits observed in Shank3 KO mouse model

Authors: *B. GIRARD, B. REDON, S. MUSARDO, C. BELLONE;
Univ. of Geneva, Geneva, Switzerland

Abstract: Maladaptive social behaviors are the core characteristics of autism spectrum disorders (ASD) but the underlying mechanisms underlying these deficits are still largely unknown. We have previously characterized a novel operant task to test social motivation in mice and we showed that Dopamine (DA) neurons from the ventral tegmental area (VTA) are activated during social interaction and signal social prediction error. Here we use Shank3 knockout as a mouse model, to test the hypothesis that social deficits are characterized by deficits in motivation to interact with conspecific and that aberrant activity of VTA DA neurons is at the origin of these behavioural alterations. We observed differences in social reinforcement learning accompanied by reduced DA neuron activity between Shank3 KO mice compared to control mice. Close-loop optogenetic strategies to overcome behavioural deficits by restoring DA neurons activity are currently under investigation. These findings pave the way to understand how deficits in VTA-DA neuron activity could contribute to social impairment observed in other neuropsychiatric disorders.

Disclosures: B. Girard: None. **B. Redon:** None. **S. Musardo:** None. **C. Bellone:** None.

Poster

650. Social Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 650.13

Title: WITHDRAWN

Poster

650. Social Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 650.14

Topic: G.03. Motivation

Support: MH119422

Title: Serotonin modulates social responses to stressed conspecifics via insular 5-HT_{2C} receptors in male and female rats

Authors: *A. J. NG, B. H. BRADY, L. K. VINCELETTE, J. P. CHRISTIANSON;
Boston Col., Chestnut Hill, MA

Abstract: Social interaction allows for the transfer of affective states among community members in a phenomenon referred to as "emotional contagion." The behaviors and expressions of individuals experiencing pain and fear can evoke anxiety-like states in observers which shape subsequent social interactions. We hypothesized that these social reactions to stressed individuals engage the serotonergic dorsal raphe nucleus (DRN) which promotes anxiety-like behavior via postsynaptic action of serotonin at serotonin 2C (5-HT_{2C}) receptors in the forebrain. First, we inhibited the DRN by administering an agonist (8-OH-DPAT, 1µg in 0.5µL) for the inhibitory 5-HT_{1A} autoreceptors which silence 5-HT neuronal activity via G-protein coupled inward rectifying potassium channels. In adult males, 8-OH-DPAT prevented the approach and avoidance, respectively, of stressed juvenile (PN30) or stressed adult (PN50) conspecifics in the social affective preference test. Similarly, systemic administration of a 5-HT_{2C} receptor antagonist (SB242084, 10mg/kg, i.p.) prevented approach and avoidance of stressed conspecifics. Seeking a locus of 5-HT_{2C} action, we considered the posterior insular cortex which is critical for social affective behaviors. Retrograde tracing confirmed that ≈10% of DRN neurons project to the insular cortex and preliminary analysis indicates that ≈20% of these projections contain the rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase (TPH2). SB242084 administered directly into the insular cortex (5µM bilaterally in 0.5µL) in both adult and female test rats also interfered with the typical approach and avoidance toward stressed juveniles or adults, respectively. These data suggest that interactions with stressed others drives activity in serotonergic neurons in the DRN that project to the insula and that serotonin modulates social affective decision-making via action at 5-HT_{2C} receptors.

Disclosures: A.J. Ng: None. **B.H. Brady:** None. **L.K. Vincelette:** None. **J.P. Christianson:** None.

Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 651.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Trinity University Murchison Summer Undergraduate Research Fellowship

Title: The effects of oxytocin administration directly into the dorsal hippocampus on cocaine seeking behavior in male and female rats

Authors: *C. MOYE[1], K.-C. LEONG[2];
[2]Dept. of Psychology, [1]Trinity Univ., San Antonio, TX

Abstract: Cocaine drug abuse dramatically affects individuals' lives nationwide. The 2020 National Survey on Drug Use and Health (NSDUH) estimated that 1 in 5 drug overdose deaths involve cocaine. Although peripheral oxytocin administration has been shown to diminish cocaine seeking behavior in rats, more research must be conducted to establish which brain structures are involved in this effect. Cocaine conditioned place preference (CPP) paradigms offer the ability to measure cocaine-seeking behavior in addition to cocaine's motivating effects. Through this paradigm, context-drug associations are formed that subsequently influence behavioral preference for the drug-associated context. Given the robust expression of oxytocin receptors (OXTRs) in the dorsal hippocampus (dHC), the present study sought to compare the potential benefits of oxytocin administration directly into the dHC on expression of cocaine CPP. After rats completed conditioning trials in which cocaine was paired with a context, they were administered either oxytocin or saline into the dHC and allowed to freely choose in which context to spend time. Our results revealed that while saline-administered male and female rats successfully displayed cocaine CPP, oxytocin administration directly into the dHC produced robust effects on cocaine seeking behavior in male and female rats. While the dHC has long been established as a key structure necessary for CPP, these findings highlight the potential mechanism through which oxytocin may be attenuating cocaine-context associations in a CPP paradigm in males and females.

Disclosures: C. Moye: None. **K. Leong:** None.

Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 651.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA042792
NIDA Grant DA026994

Title: Investigation of the necessity and specificity of the dmPFC cocaine seeking ensemble

Authors: *S. LIU[1], C. M. OLSEN[2];
[1]Med. Col. of Wisconsin, Wauwatosa, WI; [2]Neurosci. Res. Ctr., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Cocaine use disorder is a chronic and relapsing neuropsychiatric disorder characterized by a strong propensity for relapse upon re-exposure to a previously cocaine-

associated environment. The dorsal medial prefrontal cortex (dmPFC) is a critical node in the mesocorticolimbic system related to cue-induced cocaine craving and seeking. There is evidence that learned associations between cues and drug seeking behavior are encoded by specific ensemble of neurons sparsely scattered throughout the dmPFC. Thus, we explored the necessity and specificity of the cocaine seeking ensemble in the dmPFC and hypothesized that inhibition of dmPFC cocaine seeking ensembles inhibits cocaine seeking memory retrieval, and these ensembles are not involved in fear conditioning memory retrieval, which is also mediated by the dmPFC. We tested this hypothesis by co-injection of viruses expressing TRE-Cre and a cre-dependent inhibitory PSAM-GlyR into the dmPFC of male and female mice to enable "tagging" of ensemble neurons with an inhibitory chemogenetic receptor. After stereotaxic and jugular catheterization surgery, mice were trained to self-administer cocaine (0.5 mg/kg) for 14 days. After 7 days forced abstinence, a 2-hour drug seeking session was performed and the ensemble was tagged. After another 14 days abstinence, mice received ligand (uPSEM792s) 30 minutes before the second drug seeking session for activation of the chemogenetic tools. 3 days after the second seeking session, mice received tone-shock associative learning in the fear conditioning training, and a context test and cued test were performed 24 hours after the training. The uPSEM792s ligand was given 30 minutes before each test. We compared these two seeking sessions and found that inhibition of the cocaine seeking ensemble suppressed cocaine seeking. We also quantified the freezing effect and found that suppression of the cocaine seeking ensemble did not affect fear memory retrieval. These results indicated that the dmPFC cocaine seeking ensemble is necessary for context- and cue-induced seeking memory retrieval, but these ensemble neurons are only specific to cocaine seeking, no effect on fear conditioning memory retrieval also regulated by dmPFC.

Disclosures: S. Liu: None. **C.M. Olsen:** None.

Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 651.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA grant R00DA039991

Title: Neuroactivational correlates of psychosocial stress-induced cocaine seeking in male and female rats

Authors: *N. M. HINDS[1], I. D. WOJTAS[1], D. M. PULLEY[1], S. J. MCDONALD[1], S. DE GUZMAN[2], N. E. HUBBARD[1], C. M. KULNICK-SOPER[1], J. J. DEBSKI[1], B. PATEL[1], C. SPENCER[1], D. F. MANVICH[1];
[1]Deptarment of Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ;
[2]Temple Univ., Philadelphia, PA

Abstract: A challenging feature of cocaine abuse is its high risk for relapse. Psychosocial stressors are well-established to promote drug craving in humans but have rarely been employed in preclinical models of drug relapse. Consequently, the underlying neural circuitry by which these stressors drive cocaine seeking has not yet been thoroughly explored. This study aimed to identify brain regions recruited during psychosocial stress-induced cocaine seeking in rats using a novel rodent model of relapse. Adult male and female Long-Evans rats were trained to self-administer cocaine (0.5 mg/kg/inf, i.v.) in 2-h daily sessions for 20 d. On days 11, 14, 17, and 20, a discrete tactile cue was present in the operant chamber, and these sessions were immediately followed by social defeat stress (SDS; n=16, 8/sex), nonsocial footshock stress (FS; n=12, 6/sex), or a no-stress control condition (n=12, 6/sex). Beginning on day 21, animals underwent extinction training during which lever-presses were not reinforced. Once responding was extinguished, rats were re-exposed to the tactile cue that signaled their assigned stress/no-stress stimulus and reinstatement of cocaine seeking was measured for 2 h under extinction conditions. Immediately after the reinstatement test, animals were sacrificed, and brains collected and processed for c-Fos expression via immunohistochemistry. All groups exhibited cocaine-seeking behavior at reinstatement test, but the effect size was largest in the SDS and FS groups. Fos expression analyses revealed that neural activity in the prelimbic prefrontal cortex, the ventral tegmental area, and the rostral aspect of the periaqueductal gray (rPAG) significantly and positively correlated with cocaine seeking magnitude. Further analysis indicated that activity within the lateral subdomain of the rPAG (rPAGl) was uniquely correlated with reinstatement magnitude in the SDS group. Lastly, the association between rPAGl activation and cocaine seeking within the SDS group was selectively driven by male subjects. These results suggest that psychosocial stress may trigger cocaine seeking via a unique neural network that involves the rPAGl in a sex-dependent manner.

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Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 651.04

Topic: G.09. Drugs of Abuse and Addiction

Title: The network-level correlates of cocaine use disorder

Authors: *J. A. RICARD[1], L. LABACHE[1], S. CHOPRA[1], E. DHAMALA[1], N. G. HARNETT[2,3], G. JONES[4], S. W. YIP[1], A. J. HOLMES[1];
[1]Yale Univ., New Haven, CT; [2]McLean Hosp., Belmont, MA; [3]Harvard Med. Sch., Boston, MA; [4]Harvard Univ., Cambridge, MA

Abstract: Alterations in brain function and structure are reported among individuals with substance use disorders (SUD). Prior work in this domain has focused on cortico-striatal circuits in the development and maintenance of SUD. In healthy populations, individual differences in behavior and cognition are reflected in variability across the collective set of functional brain connections (functional connectome). Moreover, among individuals in treatment for SUD, differences in connectome-based profiles robustly predict treatment outcomes. Despite this, the connectomic profile of individuals with SUDs remains not yet fully understood. Using the Mexican magnetic resonance imaging dataset of patients with cocaine use disorder (SUDMEX), an open-access dataset of individuals with cocaine use disorder (CUD, n=72) and healthy matched controls (n=134), we characterize brain network changes using resting-state functional connectivity. To examine the functional network interactions affected by CUD, we computed whole-brain functional coupling matrices for each participant. Using the network-based-statistic, we characterize whole-brain functional connectivity differences between individuals with and without CUD. Our analyses revealed a widespread network of affected connections between individuals with CUD and matched controls, extending across the functional connectome (8274 edges; $p<0.05$ FWE-corrected). We find hyperconnectivity between subcortical-ventral attention network and within the visual network in individuals with CUD. At a regional level, mid-temporal, fusiform gyrus, and post-cingulum regions emerged as areas of particularly increased hyperconnectivity. By contrast, limbic-visual and subcortical-dorsal attention networks exhibited patterns of hypo-connectivity. At a regional level, this hypoconnectivity was prominent within precuneus, angular, mid frontal, and caudate nuclei. Taken together, these results provide evidence for a wide-spread network-level disruptions across the functional connectome among individuals with CUD.

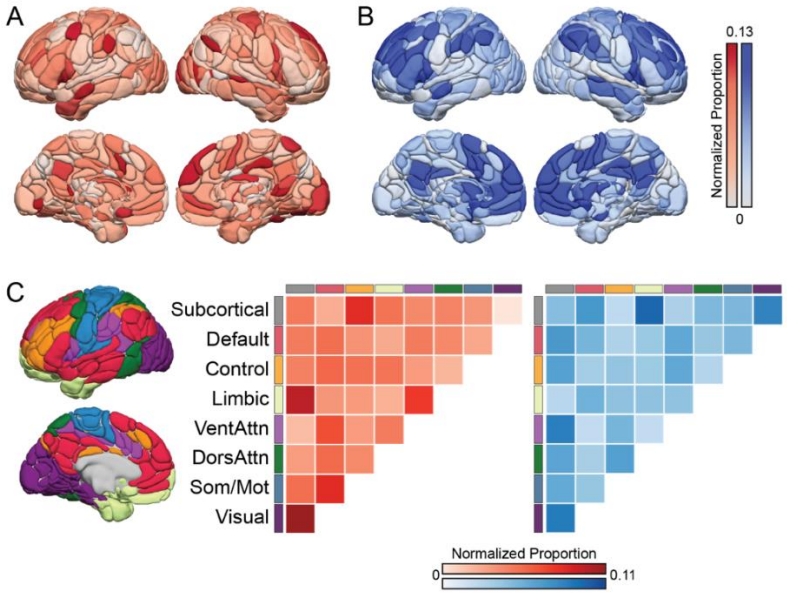

Figure 1: Normalized proportions of functional edges showing a A) significant increase in connectivity for controls compared to patients (red). The darker colors indicate a greater normalized proportion of significant edges. B) significant decrease in connectivity for controls compared to patients (blue). C) Colors reflect regions estimated to be within the same network according to the 7 networks of Yeo et al. (Yeo et al. 2011) overlaid across all available parcels within the Atlas of Intrinsic Connectivity of Homotopic Areas (AICHA atlas) (Joliot et al. 2015). 2D-matrix displays at the network level the normalized proportion of edges with significantly increased connectivity in controls than in patients (in red), and the normalized proportion of edges with significantly reduced connectivity in controls relative to patient (in blue).

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Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 651.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Speaking for five minutes about the positive consequences of abstinence predicts drug use severity a year later in cocaine addiction

Authors: *C. AGURTO[1], G. A. CECCHI[1], E. K. EYIGOZ[1], R. NOREL[1], N. ALIA-KLEIN[2], R. Z. GOLDSTEIN[2,3];
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Abstract: To date, the effects of cocaine addiction on human brain function and behavior, especially as assessed naturalistically using language, are not fully understood. In this study we evaluated the semantic context of speaking about the consequences of cocaine use in initially abstinent treatment-seeking participants with cocaine use disorder. We hypothesized that drug-relevant speech-based features would be predictive of clinical drug use outcomes at a 1-year follow-up. Twenty-seven individuals (age: 52.3 +/- 5.8 years, sex: 20M/7F) were studied at a drug free baseline and again a year later. In each of these sessions, participants described the positive consequences of being abstinent and the negative consequences of consuming cocaine on many aspects of their life (e.g., relationships) for about 5 minutes for each topic. Speech recordings were transcribed automatically using the Watson Speech to text software. To analyze the language structure in a meaningful representation, we used the roBERTa embedding method, which captures context (and not just single words). Specifically, we computed the semantic similarity of the speech with select phrases related to cocaine (selected from standard clinical tools) or quality of life (using the WHO assessment). We used the first baseline session to predict withdrawal, craving and abstinence 12 months later. Models using baseline demographic and clinical variables (age, gender, education, age at drug use onset, years of regular drug use, and addiction severity) were also generated for comparison purposes. Prediction performance was evaluated using the Spearman rank coefficient (r) and results were cross-validated using 10-fold. Results showed that speech models ($r>0.60$) were superior to the comparison models ($r<=0.05$) in accurately predicting withdrawal ($r=0.74$), craving ($r=0.61$), and abstinence ($r=0.65$). We also found that among the speech models, the features generated using cocaine related phrases during speech about the positive consequences of abstinence were more predictive of the future drug use severity measures than the features generated using the quality of life phrases or during speaking about the negative consequences of drug use. These results show for the first time that five minutes of naturalistic free speech, which is inexpensive and readily available as a tool, in initially abstinent treatment-seeking individuals with substance use disorder can predict drug use

outcomes. In general, results support the use of such ecologically valid objective measures over demographics and common drug use measures in optimizing prediction of longitudinal clinical trajectories in drug addiction.

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Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 651.06

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Title: Brain structural and functional alteration in the cocaine self-administered common marmoset

Authors: ***S. GU**[1], S. YOON[2], J. LEE[3], H. EOM[3], J. GIM[1], S. KIM[4], E. YU[1], A. KIM[1], J. CHOI[3], Y. YI[3], J. SON[3], T. KIM[5], C.-W. PARK[1], J. HONG[1], Y.-S. JUNG[4], D. LEE[3], J. YUN[1]; [1]Col. of Pharm., Chungbuk Natl. Univ., Cheongju-si, Korea, Republic of; [2]Col. of Korean Med., Daegu Haany Univ., Daegu, Korea, Republic of; [3]Osong Med. Innovation Fndn., Cheongju-si, Korea, Republic of; [4]Pusan Natl. Univ., Busan, Korea, Republic of; [5]Catholic Univ. of Daegu, Gyeongsan-si, Korea, Republic of

Abstract: Brain dysfunction and structural changes are related with repetitive and stereotyped behaviors, cocaine-induced rapid head movement in common marmoset (Callithrix jacchus) can serve as a model for stereotype. In this study, we studied effects of binge cocaine administration on the marmoset behaviors, brain structural and functional alteration. Cocaine was self-administered (SA) intravenously to adult marmosets for 30 days. After the last cocaine administration, the behaviors were observed in freely moving marmosets for 2 h. We performed resting-state functional magnetic resonance imaging (rs-fMRI) to elucidate resting-state functional connectivity (rsFC), and metabolic profiles in the plasma. A total of 30.45 mg/kg of cocaine injected for one month induced repetitive stereotyped behaviors, such as rapid head movements. We confirmed that cocaine administration changed the brain connectivity at 23 areas including the cortex, and nucleus accumbens, and availability of cannabinoid receptor type 1 and expression of glutamate decarboxylase in the cortex, and glutamine/glutamate metabolism in the plasma. Furthermore, cocaine reduced white matter structure in the corpus callosum. In proteomics study, level of dihydropyrimidinase-related protein2 (DPYSL2) that is related with

axon development and guidance regulation molecules are most altered in cocaine treated marmoset acute brain slice, and protein expression of DPYSL2 tended to decrease by treatment of cocaine. Interestingly, gene-gene interaction analysis revealed that crystallin alpha B (CRYAB), a chaperone expressed in oligodendrocyte is associated with DPYSL2, and we also confirmed that cocaine significantly induced expression of CRYAB and p-CRYAB in oligodendrocytes-positive cells in the cortex. Meanwhile, to develop a deep learning-based automatic striatal dopamine transporter (DAT) identification algorithm, we used the ^{18}F-FP-CIT PET images of cocaine SA marmosets and the U-net neural network. As a result, significant strong negative correlation between SA data and striatal DAT availability on PET image was shown, and the U-NET based segmentation for striatal DAT showed 0.96 of accuracy, 0.84 of intersection over union and 0.91of Dice. Taken together, the study suggests that the cocaine chronic SA induced disturbance in the cortex connectivity and modification in the corpus callosum white matter via regulation of endogenous cannabinoid system and GABAergic neurotransmission, axon development, moreover, an automatic identification algorithm for DAT could be helpful for image analysis for research to pharmacological researchers who are not imaging experts.

Disclosures: **S. Gu:** None. **S. Yoon:** None. **J. Lee:** None. **H. Eom:** None. **J. Gim:** None. **S. Kim:** None. **E. Yu:** None. **A. Kim:** None. **J. Choi:** None. **Y. Yi:** None. **J. Son:** None. **T. Kim:** None. **C. Park:** None. **J. Hong:** None. **Y. Jung:** None. **D. Lee:** None. **J. Yun:** None.

Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

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Program #/Poster #: 651.07

Topic: G.09. Drugs of Abuse and Addiction

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Title: Effects of hM4Di DREADD activation in locus coeruleus of rats during auditory cocaine conditioning and cue-orienting behavior

Authors: *M. PRESKER[1], G. ASTON-JONES[2];
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Abstract: Cocaine-induced changes in attention systems may underlie reactivity to drug-cues in cocaine addiction. Behavioral reactivity and neural encoding of drug cues is a primary feature of addiction that contributes to relapse vulnerability, a primary therapeutic target in addiction treatment. The locus coeruleus (LC) is a noradrenergic brainstem nucleus that projects throughout the neocortex and modulates attention. LC regulates drug-cue memories and stress-induced drug seeking but it is not clear what role the LC plays in encoding drug-cue salience. We developed a behavioral paradigm to examine the effects of auditory cocaine cues on attention

during natural reward motivated behavior. First, rats were trained to nose poke for water on a variable-interval 10s schedule (VI10) during daily sessions. Rats then underwent daily conditioning (6d) to associate one auditory cue with cocaine (20mg/kg; intraperitoneal) and another with saline. After conditioning, the effect of the cues on stimulus orienting and responding for water was measured. Cocaine cues produced a generalized positive transfer effect to increase water self-administration. To test the causal role of LC, we used activated hM4Di in LC during either the acquisition of the drug-tone association (during conditioning) or during the expression (during tone testing). TH-cre+ rats (N=36) received injections of virus to express the hM4Di DREADD receptor or GFP in LC (n=18 each). Rats were trained on the water task and split into acquisition and expression groups. Rats in the acquisition group underwent conditioning as described except that cocaine injections were preceded by 30min with clozapine-N-oxide (1mg/kg; IP) to activate the hM4Di receptors in LC prior to tone conditioning sessions, and then tested normally. Rats in the expression group underwent conditioning as usual but received CNO pretreatment prior to tone testing sessions during water self-administration behavior. Behavior during the conditioning and expression tests was analyzed using between subjects to determine the effects of LC inhibition by hM4Di. Activating LC-hM4Di during the acquisition of an auditory cocaine memory reduced drug cue salience (decreased general transfer effect) compared to GFP. Activating LC-hM4Di during the expression of an auditory cocaine memory increased drug cue salience (increased general transfer effect) compared to GFP. Further analyses are underway to record the activity of LC neurons during cues.

Disclosures: M. Presker: None. **G. Aston-Jones:** None.

Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

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Program #/Poster #: 651.08

Topic: G.09. Drugs of Abuse and Addiction

Support: 2021R1A2C2008083

Title: Linking of NMDA receptors and mGluR5 in the nucleus accumbens core to repeated cocaine-induced 50-kHz ultrasonic vocalization in rats

Authors: *S. SOHN, S. KIM, E. S. CHOE;
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Abstract: Rats express a positive emotional state by emitting 50-kHz ultrasonic vocalization (USV) calls in response to drug exposure. This study demonstrated the linking of glutamate receptors in the nucleus accumbens (NAc) to vocal expression of 50-kHz USV calls after repeated cocaine administration in freely moving rats. Repeated systemic injections of cocaine (20 mg/kg/day, i.p.) for seven consecutive days increased the number of 50-kHz USV calls. Intra-NAc core infusion of the broad-glutamate receptor antagonist, γDGG (50 nmol/side),

decreased the repeated cocaine-induced increase in the number of 50-kHz USV calls. Intra-NAc core infusion of the N-methyl-D-aspartate (NMDA) receptor antagonist, MK801 (2 nmol/side), but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or kainic acid receptor antagonist, CNQX disodium salt (2 nmol/side), decreased the number of 50-kHz USV calls that had been elevated by repeated exposure to cocaine. Intra-NAc core infusion of the group I metabotropic glutamate receptor subtype 5 (mGluR5), MPEP (0.5 nmol/side), MTEP (15 nmol/side), and inositol-1,4,5-trisphosphate receptor blocker, xestospongin C (0.004 nmol/side), decreased the cocaine-induced increase in the number of USV calls. These data suggest that the NMDA receptors- and mGluR5-dependent increase in intracellular Ca^{2+} concentrations in the NAc core is linked to a positive emotional state after repeated exposure to cocaine in rats.

Disclosures: **S. Sohn:** None. **S. Kim:** None. **E.S. Choe:** None.

Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

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Title: Effects of oral Lactobacillus spp. pretreatment on sensitized response induced by repeated smoked cocaine and the influence on gut microbiota structure

Authors: *S. FABIUS[1], J. URBANAVICIUS[1], S. FERNANDEZ-CIGANDA[3,2], J. PRIETO[4], J. LOZANO[2], C. PICCINI[2], P. ZUNINO[2], M. C. SCORZA[1];
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Abstract: Emerging studies highlight the potential role of intestinal microbiota (IM) modulation in brain disorders; however, studies addressing its involvement in substance use disorder (SUD) are limited. Cocaine systemic administration in rodents induces gut dysbiosis and chronic antibiotics enhance its rewarding property. Accordingly, we demonstrated that repeated exposure to volatilized cocaine (14 days) alters the IM structure and diversity in rats, leading to hypothesized that IM modulation by probiotic bacteria can attenuate cocaine effects. The present study aims to evaluate the role of the IM modulation on the changes induced by the chronic administration of smokable cocaine on locomotor sensitization, and IM structure. Adult male Wistar rats were administered via oral syringe-feeding with a bacterial mixture of three probiotic Lactobacillus strains (*L. johnsonii* ATCC 33200; *L. rhamnosus* GG ATCC 53103; *L. reuteri*

ATCC 23272; 1x10E8 CFU in 0.5 ml) or vehicle (skim milk) for 28 days. From day 22 to 28, rats were also daily exposed to cocaine (7 days/25 mg) by pulmonary inhalation, and locomotor activity in the open field was assessed. Fecal samples were collected at different time points and processed for DNA extraction, sequencing and posterior microbiota analysis. Behavioral results showed that oral bacteria administration did not *per se* affect locomotor activity. In cocaine-exposed rats we observed a progressive stimulant effect (locomotor sensitization) from day 1 to 5 of cocaine, and decreased in days 6 and 7. Bacteria administration did not prevent cocaine sensitization, and maintained the motor activity elevated until the last day. On day 28, no significant differences were found in the microbiota structure in cocaine-exposed animals in comparison with the control group. However, animals administered with bacteria and exposed to cocaine showed differences in their IM structure compared to bacteria control group (p= 0.038), and tend to differ concerning the cocaine group (p=0.052). All these results suggest that 7 days of volatilized cocaine are not enough to significantly change IM structure, but an influence of bacterial mixture pretreatment was observed, suggesting a possible role in the sustained cocaine-stimulant effect. Altogether our findings provide information about the role of gut-brain axis in SUD. Further experiments should be done to evaluate the potential benefits of other bacterial strains for microbial-based therapeutic strategy in SUD.

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Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

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Program #/Poster #: 651.10

Topic: G.09. Drugs of Abuse and Addiction

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The Foundation of Hope (SPF)

Title: Transmembrane AMPA regulatory protein γ-8 (TARP γ-8) is required for the induction of locomotor sensitization to cocaine in mice

Authors: *C. M. WHINDLETON, J. L. HOFFMAN, J. S. LEE, E. M. HOMBERGER, S. P. FACCIDOMO, C. W. HODGE;
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Abstract: *Background:* Behavioral sensitization is the escalated locomotor response that occurs from repeated administration of a drug over time— a phenomenon linked to the neuronal plasticity associated with drug reward and reinforcement, and a common animal model of substance use disorders. Repeated drug use leads to synaptic adaptations in the ventral tegmental area and nucleus accumbens (Nacb) by potentially altering synaptic transmission and increasing the projection strengths to these areas. This shift occurs, in part, through increased expression of

the glutamatergic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPARs). Repeated administration of cocaine increases the expression of the AMPAR GluA1 subunit, increasing synaptic strength and behavioral plasticity. Transmembrane AMPA regulatory proteins (TARPs) bind to GluA1 to regulate receptor trafficking and synaptic plasticity. Interestingly, the TARP subtype, TARP γ-8, is highly restricted to Nacb-projecting regions such as the frontal cortex, hippocampus, and basolateral amygdala, important regions for conditioned drug use. *Objective:* Due to TARP γ-8's regionally selective expression and its potential in regulating synaptic excitation and AMPAR expression, we sought to test the effects of TARP γ-8 on cocaine sensitization. *Methods:* 12-18-week-old, C57BL/6J male and female TARP γ-8 wildtype (WT; +/+) and knockout mice (KO; -/-) were tested for behavioral sensitization to cocaine. Mice were acclimated to locomotor chambers for 30min sessions for 3-consecutive days prior to the experiment to habituate to the open field to reduce novelty. Separate groups of WT and KO mice were then injected with 10 mg/kg, i.p. cocaine or saline, for 7 consecutive days. On days 1, 4, and 7 mice were placed into locomotor chambers after injections for 30min to assess locomotor activity. *Results*: KO mice showed greater initial activity in the open field arena but habituated to similar levels as WT mice over time. In the WT mice, repeated injections of cocaine significantly increased their locomotor response to cocaine confirming a robust induction of behavioral sensitization. Notably, this locomotor response was significantly blunted in both male and female KO mice, with small differences between sexes. *Conclusion:* Our data suggest that TARP-γ8 may be required for the full induction of behavioral sensitization to cocaine. Additionally, due to the anatomical specificity of TARP γ-8 expression, this work suggests that TARP γ-8 may represent a novel, druggable target for treatment of substance use disorder.

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Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

Location: SDCC Halls B-H

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA DA031695
Mid-Atlantic Neuroscience Diversity Scholars (MINDS)

Title: Do rats that have previously self-administered cocaine delay gratification?

Authors: *N. KANG[1], H. PRIBUT[1], M. R. ROESCH[2];
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Abstract: Exposure to drugs of abuse produces impairments in studies of reversal learning, delay discounting, and response inhibition. While these studies contribute to the understanding of decision-making and how it is impaired by drugs of abuse, they do not fully capture how decision-making impacts the ability to delay gratification for greater long-term benefit. To address this issue, we used a diminishing returns task to study decision-making behavior from rats that had previously self-administered cocaine, as compared to control rats that were rewarded with sugar pellets. This task is designed to test the ability of the rat to choose to delay gratification in the short term to obtain more reward over the course of the entire behavioral session. Antidotally, this is similar to an addict choosing to take the time to go to rehab to improve their life in the long run. In this task, a total of 22 rats were presented with two choices, with 11 rats in the experimental group (6M; 5F) and 11 rats in the control group (5M, 6F). One choice had a fixed amount of time delay needed to obtain reward (FD), while the other choice had a progressive delay (PD) that started at 0s and progressively increased by 1 s each time the PD option was selected. During the "reset" variation of the task, rats could choose the FD option to reset the time delay associated with the PD option, while in the "no reset" variation, the PD delay continued to increase regardless of FD selection. During reset sessions, the cocaine and control rats selected the PD trough at similar rates ($F(1,40) = 2.1$, $p = 0.155$). Similarly, the cocaine and control rats selected the PD trough at similar rates during no-reset sessions ($F(1,50) = 0.21$, $p = 0.647$). Additionally, consistent with previous results, we found that prior cocaine exposure reduced rats' overall preference for the PD option in post-reversal no reset sessions ($t(48) = -2.021$, $p = 0.049$), suggesting that cocaine exposure made rats more sensitive to the increasing delay. Surprisingly, however, we found that rats that had self-administered cocaine adapted behavior during reset sessions by delaying gratification to obtain more reward in the long run, similarly to control rats. That is, both groups selected the FD lever with a longer delay to reset the PD delay back to zero prior to the equality point, thus achieving more reward over the course of the session. Although these findings do not support our hypotheses, they still support previous work demonstrating general deficits in reversal learning and sensitivity to delays to reward after previous drug use. Future versions should consider progressive ratio schedules instead of manipulations of delay when examining diminishing returns.

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Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

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Program #/Poster #: 651.12

Topic: G.09. Drugs of Abuse and Addiction

Support: Arnold and Mabel Beckman Foundation

Title: Males, but not females, prefer a socially-paired context over a cocaine-paired context in a conditioned place preference paradigm

Authors: *E. LORENZ[1], K.-C. LEONG[2];
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Abstract: Cocaine is involved in nearly 1 in 5 overdose deaths in the United States. Although these death rates decreased between 2006 and 2012, they have begun to climb again, increasing by 9% in 2019 (CDC). Recent evidence demonstrates that cocaine use is diminished in individuals with strong social support systems, suggesting that social reward may combat the rewarding effects of cocaine. Sex differences are observed in both drug seeking behavior and social interaction. To examine the competing nature of cocaine and social reward, male and female rats were trained in a conditioned place preference paradigm in which one context was paired with cocaine (Males: 15 mg/kg, i.p; Females: 5 mg/kg, i.p.) while another was paired with social reward (i.e. social interaction). Briefly, conditioning occurred over 8 days alternating between the two neutral contexts and drug administration was counterbalanced within the groups. Results revealed that during test day male rats showed preference for the conspecific-paired context relative to the cocaine-paired context. The opposite effect was seen in female rats, where preference was shown for the cocaine-paired context over the conspecific-paired context. The neuropeptide oxytocin (OXT) is implicated in both reward and social processes. Therefore we also examined the effect of OXT administration during conditioning in female rats. Specifically, OXT (1 mg/kg; i.p.) was administered 30 minutes prior to all 8 conditioning trials. On test, results revealed that OXT administration during conditioning diminished preference for the cocaine-paired context. While these results indicate that OXT may influence cocaine-associated and conspecific-associated contextual preference, future research needs to be carried out to determine whether OXT administration is specifically attenuating cocaine-associated reward or enhancing conspecific-associated reward.

Disclosures: E. Lorenz: None. **K. Leong:** None.

Poster

651. Neural and Behavioral Variables Associated with Cocaine Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA044297
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Title: Isoform-selective PI3-kinase inhibition confers partial resilience to cocaine cessation-induced anxiety-like behavior

Authors: *B. R. BARBEE[1,2,3,4], S. L. GOURLEY[1,2,3,4];
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Abstract: Phosphoinositide 3-kinase (PI3K) is a multi-subunit signaling complex that phosphorylates phosphoinositides, membrane-embedded second messengers that are critical for synaptic and structural plasticity of neurons. Cocaine potentiates PI3K-Akt-mTOR cascade activity, and this activation persists beyond the period of drug exposure. The PI3K p110β isoform is neuronally enriched and able to control PI3K signal propagation, allowing for manipulation of PI3K activity in a more targeted manner than broad-spectrum PI3K inhibition. Cessation of cocaine use triggers anxiety-like behavior in humans and rodent models, and anxiety can be a causal factor in relapse. Here, we used viral-mediated gene silencing to reduce expression of p110β in the dorsomedial prefrontal cortex (dmPFC). Isoform-selective PI3K inhibition mitigated anxiety-like behavior triggered by acute cocaine. Interestingly, however, a history of repeated cocaine exposure occluded this resilience, presenting an opportunity to compare immediate-early gene expression between cocaine-vulnerable and cocaine-resilient mice. We examined 22 brain regions and found that resilient mice - those displaying less anxiety-like behavior - displayed lower immediate-early gene expression in the claustrum. We next found that chemogenetic stimulation of the claustrum induced anxiety-like behavior, and that chemogenetic inhibition of claustrocortical projections attenuated cocaine abstinence-elicited anxiety-like behavior. Further, corticoclaustral and claustrocortical projection inhibition mitigated cue reactivity following oral cocaine self-administration. Our findings suggest that isoform-selective PI3K inhibition mitigates cocaine cessation-elicited anxiety-like behavior, likely via coordinated brain regions and circuits.

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Poster

652. Fentanyl Seeking

Location: SDCC Halls B-H

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Program #/Poster #: 652.01

Topic: G.09. Drugs of Abuse and Addiction

Support: P50 DA04412

Title: Influence of fentanyl vapor self-administration conditioning paradigm on drug motivation following abstinence

Authors: *M. CENTENO[1], J. COX[1], R. JABAKHANJI[1], A. BRINK[1], S. E. CERMAK[1], A. APKARIAN[1,2];
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Abstract: Fentanyl vaping (Moussawi et al, 2021) provides a powerful context for the study of opioid self-administration in chronic pain. However, we have limited knowledge about how

different conditioning paradigms alter the motivation to seek fentanyl after a period of abstinence or how this may be affected by chronic pain. To address this question, we tested how different fentanyl vapor self-administration conditioning paradigms affected drug motivation following abstinence, as well as discrimination between active and inactive nose pokes (69 mice, 43 female, 24 with spared nerve injury, 21 with sham surgery, 23 healthy). We varied fentanyl-associated cues (visual or visual+auditory conditioned stimuli), reward schedules (fixed ratio, intermittent access), fentanyl dose (from 2.5mg to 30 mg/ml per delivery) and duration of abstinence. Our results show that the vaping conditioning paradigm affects opioid self-administration and drug seeking behavior. In particular, the type of conditioned stimulus affected discrimination between active and inactive nose pokes, while the reward schedule affected drug seeking after abstinence. Mice trained on an intermittent access paradigm showed lower levels of fentanyl seeking after 2 weeks of abstinence compared to mice trained only on fixed ratio. With increased durations of abstinence, however, responding increased in intermittent access mice but decreased in fixed ratio mice. This suggests that the details of the self-administration protocol affect drug craving and shows that fentanyl vaping is a powerful tool that may allow dissection of how distinct facets of chronic pain affect opioid seeking.

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Poster

652. Fentanyl Seeking

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Program #/Poster #: 652.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Single-trial fentanyl sensitization and conditioned activity in juvenile rats

Authors: D. L. SANCHEZ, D. J. GONZALEZ, J. A. TAYLOR, K. J. ABELLAR, *C. A. CRAWFORD;
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Abstract: Over a 20-year period in the US, the annual number of opioid painkiller prescriptions rose 288% and this increase was mirrored by a four-fold rise in treatment admissions. Drug overdose rates increased the most for persons aged 25-34 years but there has been a sharp increase in the number of opioid overdoses in juveniles and young adults in recent years. Most of these overdoses were primarily a result of the rise in the availability of synthetic prescription opioids like fentanyl. Thus, the goal of the current study was to assess the development of abuse liability of fentanyl using a one-trial behavioral sensitization paradigm. In this experiment, postnatal day (PD) 19 male and female Sprague-Dawley rats were injected once with fentanyl (50, 100, 200 or 400 µg/kg, sc) or saline and placed immediately in locomotor activity chambers for 60 min. After a 48-h abstinence period, all rats were injected with fentanyl (0 or 100 µg/kg, sc) and placed in the locomotor activity chambers for 120 min. On the first injection day (i.e., the

pretreatment day), fentanyl reduced the locomotor activity of all groups as rats injected with fentanyl (100, 200 and 400 µg/kg) were less active than rats treated with saline. On the second injection day (i.e., the test day) rats pretreated with fentanyl (100, 200 and 400 µg/kg) and challenged with 100 µg/kg exhibited more locomotor activity than rats pretreated with saline. Interestingly, rats pretreated with fentanyl (400 µg/kg) and challenged with saline also had greater distance traveled than saline pretreated rats despite having decreased activity on the pretreatment day. In summary, juvenile rats show both behavioral sensitization and conditioned activity after fentanyl pretreatment similar to many other drugs of abuse. These findings suggest that juvenile rats, similar to adult rats, are vulnerable to the addictive properties of fentanyl.

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Poster

652. Fentanyl Seeking

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH RCMI GRANT 12749045

Title: Early-life maternal separation sex-dependently alters fentanyl seeking in adolescence and adulthood

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[1]Biol. Sci., Kent State Univ., Stow, OH; [2]Biol. Sci., Kent State Univ., Kent, OH

Abstract: The opioid epidemic is a major health crisis in the U.S., resulting in an estimated 80,816 deaths in 2021. According to recent statistics, 1.6 million people began the non-medical use of prescription opioids in 2021, with an additional 50,000 beginning the recreational use of heroin. The susceptibility to substance abuse disorders is influenced by several factors, including traumatic childhood experiences such as parental neglect. Early Childhood Neglect (ECN) accounts for up to 75.3% of all child maltreatment cases in the US annually. Stressful and traumatic experiences during childhood, such as ECN, coincide with increased susceptibility to Opioid Use Disorder (OUD), with survivors being twice as likely to be prescribed opioids and 4.5 times more likely to develop OUD. Thus, we assessed the effects of maternal separation (MS), a potent form of ECN, on seeking behavior during adolescence and adulthood using a rat model of fentanyl-induced Conditioned Place Preference (CPP). Sprague Dawley rat pups (n = 64; male = 28, female = 36) were cross fostered at birth and received 3-hour daily MS on P2-P18 or were allowed to receive full maternal care. Rats were then assigned to adolescent (P32) or adulthood (≥ P50) place conditioning in a three-chamber apparatus for eight days under one of four conditions: 1) control - saline (1 ml/kg, s.c.), 2) control - fentanyl (5 ug/kg, s.c.), 3) MS - saline, or 4) MS - fentanyl. Extinction testing was conducted 24-hours post-conditioning for

three days during adolescence and for eight days and then weekly in adulthood until day 91 or until extinction criteria was reached. We found that MS enhanced the magnitude and persistence of fentanyl-seeking behavior in adolescent and adult males as compared to control non-MS rats ($p<0.005$). In contrast, MS impaired the formation of fentanyl-seeking behavior in adolescent and adult female rats as compared to control non-MS rats ($p<0.005$). We observed that both males and females in the maternal separation group without any drug expressed a high level of anxiety, evidenced by increased time spent in the center chamber of the CPP apparatus and less overall locomotion. Thus, MS induced a significant but sex-dependent alteration in the expression of fentanyl-seeking behavior during adolescence and adulthood. These findings are consistent with human studies on the effects of ECN on the propensity and persistence of OUD, permitting future studies on the neurobiological mechanisms by which MS alters fentanyl seeking sex-dependently later in life using this model.

Disclosures: F. Aboalrob: None. **Z.T. Knauss:** None. **D. Mueller:** None.

Poster

652. Fentanyl Seeking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 652.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA009813
UG3 Grant DA050325

Title: Effects of acute and chronic treatment with the glucagon-like peptide-1 receptor agonist, liraglutide, on cue-induced seeking and drug-induced reinstatement of fentanyl seeking in male and female rats.

Authors: L. A. URBANIK, N. K. ACHARYA, P. S. GRIGSON;
Neural and Behavioral Sci., Pennsylvania State Univ. Col. of Med., Hershey, PA

Abstract: Opioid Use Disorder (OUD) is a chronic relapsing disorder that has severe negative impacts on the individual, the family, and the community at large. In 2021, opioids contributed to nearly 80% of all drug overdose deaths in the US. This rise in opioid-related deaths coincides with a significant rise in the use of fentanyl, a synthetic opioid that is 150 times more potent than morphine. This overdose trend has spared no demographic and costs the nation an estimated $78.5 billion annually. Thus, it is imperative to better understand the underlying mechanisms of OUD in an effort to identify new treatment targets. Using animal models, studies have shown that rats readily self-administer heroin and actively seek the drug following exposure to drug-related cues, the drug itself, or stress. We have shown that treatment with the glucagon-like peptide-1 receptor (GLP-1R) agonist, liraglutide, can reduce heroin taking and seeking behavior in rats. We established an animal model of fentanyl self-administration to test whether acute and chronic treatment with liraglutide also can reduce fentanyl seeking in fentanyl-experienced male

and female rats. Rats were trained to self-administer fentanyl through an indwelling intravenous catheter for 6 hours per day for 14 days. Rats then underwent an extinction and reinstatement test. The results showed that rats readily self-administer fentanyl (2.5 ug/kg) intravenously, with marked individual differences in drug-taking behavior. As with other drugs of abuse, rats exhibited high seeking behavior when challenged with a drug-related cue or the drug itself. Here, acute treatment with the GLP-1R agonist, liraglutide (0.3 mg/kg s.c.), was found to attenuate both cue-induced fentanyl seeking and drug-induced reinstatement of fentanyl seeking at the same efficacy as the currently approved partial opioid agonist, buprenorphine. When the liraglutide dose was gradually increased over a 15-day abstinence period from 0.06 to 0.3 mg/kg, it did not reduce cue-induced fentanyl seeking; however, there was a trend for reduced drug-induced fentanyl seeking. Taken together, these data suggest there may be adaptability to chronic liraglutide treatment, but overall suggest that a known satiety signal, GLP-1, may serve as an effective non-opioid alternative for the treatment of OUD.

Disclosures: L.A. Urbanik: None. **N.K. Acharya:** None. **P.S. Grigson:** None.

Poster

652. Fentanyl Seeking

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Program #/Poster #: 652.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01

Title: Exploring the role of neuropeptide y receptor type 2 (Y2R) in fentanyl taking and seeking

Authors: *A. CAFFREY[1], R. MERKEL[2], Y. ZHANG[2], H. D. SCHMIDT[3];
[2]Biobehavioral Hlth. Sci., [1]Univ. of Pennsylvania, Philadelphia, PA; [3]Dept Psychiatry, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: There has been a dramatic increase in illicit fentanyl use in the United States over the last decade. In 2020, more than 57,000 overdose deaths involved fentanyl or fentanyl analogs, highlighting an urgent need to identify more effective treatments for fentanyl use disorder. An emerging literature indicates that glucagon-like peptide-1 receptor (GLP-1R) agonists attenuate opioid taking and seeking. However, these responses were associated with malaise-like effects in opioid-dependent rats. Recently, we showed that simultaneous activation of GLP-1Rs and Y2Rs attenuates fentanyl taking and seeking with little to no adverse effects compared to GLP-1R monotherapy. The role of Y2Rs alone in opioid taking and seeking, however, is not known. Here, we investigated that ability of the Y2R ligand PYY_{3-36} to reduce fentanyl self-administration and reinstatement. Rats were allowed to self-administer fentanyl (2.5 μg/kg, i.v.) for 21 days on a fixed-ratio 5 (FR5) schedule of reinforcement. Rats were then pretreated with vehicle or PYY_{3-36} (systemic: 50 μg/kg; intra-VTA: 0.1 and 1.0 μg/100nL) before the beginning of fentanyl self-administration test sessions. There were no effects of systemic or intra-VTA

PYY_{3-36} on fentanyl self-administration. Opioid taking was then extinguished by replacing the fentanyl solution with saline. Opioid seeking during abstinence was then elicited using an acute priming injection of fentanyl (45 μg/kg, i.p.). Prior to fentanyl-primed reinstatement test sessions, rats were pretreated with vehicle or PYY_{3-36} (systemic: 50 μg/kg; intra-VTA: 0.1 and 1.0 μg/100nL). Our preliminary findings suggest that PYY_{3-36} may reduce fentanyl seeking during abstinence. Taken together, these results indicate that Y2R agonism alone may not be sufficient to reduce fentanyl taking and seeking and further support development of dual agonists of GLP-1Rs and Y2Rs for treating opioid use disorders.

Disclosures: A. Caffrey: None. **R. Merkel:** None. **Y. Zhang:** None. **H.D. Schmidt:** None.

Poster

652. Fentanyl Seeking

Location: SDCC Halls B-H

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Program #/Poster #: 652.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH 5R21DA052101-02

Title: Crispr epigenome editing of nucleus accumbens medium spiny neuron subtype transcripts regulated by fentanyl abstinence

Authors: *M. TURNER[1], N. HARRIS[1], E. CHOI[1], R. CHANDRA[2], S. AMENT[3], M. E. FOX[4], M. LOBO[5];
[2]Virus Vector Core, [1]Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Univ. of Maryland Inst. of Genome Sci., Baltimore, MD; [4]Penn State Col. of Med., Hershey, MD; [5]Univ. of Maryland Sch. Med., Baltimore, MD

Abstract: In recent years, the opioid use epidemic has significantly impacted the financial and societal health burdens around the world, particularly the United States. This is in part due to the ease at which synthetic opioids like fentanyl are obtained resulting in an increase in opioid overdoses. Many studies indicate that disruption of mesocorticolimbic brain areas are central to understanding opioid use, dependence, and addiction. The nucleus accumbens (NAc) is a critical brain hub for altered molecular processes mediating behavioral responses to opioids and other used substances. Therefore, understanding the molecular and transcriptional programs of the neuronal subtypes within the NAc deserve considerable and critical attention. Our lab has performed cell subtype specific translatome profiling in the two NAc projection neuron subtypes- dopamine receptor 1 and 2 expressing medium spiny neurons (D1- and D2-MSNs) after prolonged abstinence from repeated fentanyl exposure. We identified distinct MSN subtype gene expression networks that are altered during fentanyl abstinence including hub genes, which are key drivers of gene expression alterations in MSN subtype specific modules. To build on this, we are developing CRISPR epigenome editing tools that will target specific hub genes in MSN subtype modules that are significantly regulated by fentanyl abstinence- 5 days 10μg/ml

fentanyl followed by 10 days of abstinence. Since most genes are down-regulated we use CRISPRa (activation) to upregulate expression of hub genes in MSN subtypes. Using a two vector adenoassociated virus (AAV) system we designed single gRNA or multiplex gRNAs, the latter targeting multiple hub genes within one module. gRNAs targeting hub genes or a lacZ control gRNA are cloned into an AAV-gRNA-nlsGFP, which allows expression of nuclear GFP. The second vector is an AAV-DIO-dCas9-VP64 containing the nuclease dead Cas9 fused to the VP64 transcriptional activation domain. We have transfected these vectors along with Cre into Neuro2A cells and demonstrated upregulation of MSN subtype fentanyl abstinence regulated hub genes. We are currently packaging these vectors into AAVs to target NAc MSN subtypes, using D1-Cre and A2A-Cre mice, during opioid exposure and abstinence to determine if upregulating hub genes can influence MSN subtype/fentanyl abstinence gene expression networks.

Disclosures: **M. Turner:** None. **N. Harris:** None. **E. Choi:** None. **R. Chandra:** None. **S. Ament:** None. **M.E. Fox:** None. **M. Lobo:** None.

Poster

652. Fentanyl Seeking

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA046532

Title: Effects of 2,5-dimethoxy-4-methylamphetamine (DOM) and 2-piperazin-1-yl-quinoline (quipazine) on fentanyl versus food choice in rhesus monkeys

Authors: *D. MAGUIRE;
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Abstract: There has been increasing interest in the potential therapeutic effects of drugs with agonist properties at serotonin (5-HT) 2A subtype receptors (e.g., psychedelics), including treatment of substance use disorders. Studying interactions between 5-HT_{2A} receptor agonists and other drugs is important for understanding potential therapeutic effects as well as adverse interactions. Direct-acting 5-HT_{2A} receptor agonists such as 2,5-dimethoxy-4-methylamphetamine (DOM) and 2-piperazin-1-yl-quinoline (quipazine) enhance some (e.g. antinociceptive) effects of opioids; however, it is unclear whether they alter the abuse-related effects of opioids. This study examined whether DOM and quipazine alter the reinforcing effects of fentanyl in rhesus monkeys (n=6) responding under a food versus drug choice procedure. Responding on one lever delivered sucrose pellets and responding on the other lever delivered intravenous infusions. In one set of experiments, fentanyl (0.1-3.2 µg/kg/infusion) versus food choice sessions were preceded by noncontingent intravenous pretreatments with DOM (0032-0.32 mg/kg), quipazine (0.32-1.0 mg/kg), naltrexone (0.032 mg/kg), or heroin (0.1 mg/kg). In

another set of experiments, fentanyl was available during choice sessions in combination with DOM (0.32-100 µg/kg/infusion) or quipazine (3.2-320 µg/kg/infusion) in varying dose ratios. Naltrexone decreased and heroin increased fentanyl choice, demonstrating sensitivity of responding to pharmacological manipulation. However, whether given as a pretreatment or available in combination with fentanyl as a mixture, neither DOM nor quipazine significantly altered fentanyl choice. These results suggest that 5-HT_{2A} receptor agonists do not enhance the reinforcing effects of opioids and, thus, will not likely enhance abuse potential.

Disclosures: D. Maguire: None.

Poster

652. Fentanyl Seeking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 652.08

Topic: G.09. Drugs of Abuse and Addiction

Support: R33DA041883

Title: Sex differences in the role of CNIH3 in opioid seeking

Authors: *T. LINTZ, H. FRYE, E. NELSON, J. DOUGHERTY, J. MORON-CONCEPCION; Washington Univ. in St. Louis, St. Louis, MO

Abstract: Cornichon homolog-3 (CNIH3) is an AMPA receptor (AMPAR) auxiliary protein that traffics AMPARs to the postsynaptic membrane and potentiates AMPAR signaling. AMPARs are key components of hippocampal synaptic plasticity and memory formation, however the role of CNIH3 in opioid-associated memory has yet to be fully elucidated. Previous research has shown that global knockout of CNIH3 in female mice impairs short-term spatial memory. A previous genome-wide association study (GWAS) comparing humans that used opioids occasionally to those that used daily has shown that single nucleotide polymorphisms in CNIH3 provide significant protection against development of opioid use disorders, particularly in women. To investigate sex differences in the role of CNIH3 in opioid use, we assessed fentanyl consumption using an operant intravenous self-administration (IVSA) approach in male and female wildtype and CNIH3 KO mice (n=10-15/group). Our results indicate that CNIH3 KO 1) impairs fentanyl IVSA acquisition in female mice, indicating impaired opioid-associated memory formation ($p<0.0001$, 1-way ANOVA with multiple comparisons) and 2) prevents the increased consumption of fentanyl per session in males observed over time in control mice ($p<0.0001$, 2-way ANOVA and post-hoc analysis). In addition, drug seeking in absence of fentanyl delivery and drug-associated cues (extinction period) is reduced in female CNIH3 KO animals ($p=0.003$, 1-way ANOVA with multiple comparisons). Furthermore, CNIH3 KO dampens drug seeking during drug cue-induced reinstatement in both female and male mice ($p<0.0001$, 2-way ANOVA and post-hoc analysis). This study, the first to identify sex-specific effects of the AMPAR auxiliary protein CNIH3 on opioid-associated memory and opioid intake,

begins to uncover the role of CNIH3 on sexually dimorphic AMPAR-dependent behavior and hippocampal synaptic plasticity.

Disclosures: T. Lintz: None. **H. Frye:** None. **E. Nelson:** None. **J. Dougherty:** None. **J. Moron-Concepcion:** None.

Poster

652. Fentanyl Seeking

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Program #/Poster #: 652.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00DA043572-03

Title: Stress Alters Opioid Abuse Susceptibility

Authors: *C. O'BRIEN, R. VEMIREDDY, D. DESAI, U. MOHAMMED, D. BARKER; Rutgers Univ., Rutgers Univ., Piscataway, NJ

Abstract: Opioids are heavily prescribed and are highly effective for treating acute pain, but not chronic pain, resulting in a large risk factor for abuse. The ability to predict specific individual susceptibility to opioid use disorder is limited, partially due to the complex comorbidity with other mental and physical illnesses. Exposure to stress and an individual's response is known to be important for the development of substance use disorders. To better understand how specific classes of behavioral responses contribute to opioid abuse susceptibility, we developed a comprehensive battery of tests for negative valence behaviors and nociception to identify individuals predisposed to opioid seeking following oral opioid self-administration. We show that mice with a history of stress do not exhibit a preference for sucrose, showed greater immobility in the forced swim task, and exhibited mechanical pain hypersensitivity when compared to controls. Relating these behaviors to future fentanyl-seeking responses, we observed that increased mechanical sensitivity was related to higher opioid preference in mice with a history of stress, but not controls. Interestingly, we discovered that, paradoxically, high sucrose preferences predicted fentanyl preference in the stress group, while lower sucrose preference, a sign of anhedonia, predicted fentanyl preference in controls. This indicates that stress can act as a modulator, shifting opioid abuse susceptibility that can be predicted by the response to stress.

Disclosures: C. O'Brien: None. **R. Vemireddy:** None. **D. Desai:** None. **U. Mohammed:** None. **D. Barker:** None.

Poster

652. Fentanyl Seeking

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Program #/Poster #: 652.10

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA035943

Title: Differential engagement of the rat central nucleus of the amygdala during phases of fentanyl self-administration.

Authors: *E. CHALOUX-PINETTE[1], X. TONG[2], K. ANANTH[2], V. SILVA[2], P. H. JANAK[2,3];
[1]Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD; [2]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [3]Dept. of Neurosci., Johns Hopkins Universtity Sch. of Med., Baltimore, MD

Abstract: The central nucleus of the amygdala (CeA) modulates motivation for natural and drug reinforcers, but the region has been less studied in self-administration models of opioid use disorders. To assess the role of the CeA in opioid self-administration, we trained male and female Sprague-Dawley rats (n=25, n=18, respectively) to self-administer the synthetic opioid, fentanyl (0.5μg/i.v. infusion), on an FR1 schedule in 2-hr daily sessions. After training for at least 28 sessions, rats maintained robust, stable intake patterns. CeA microinfusion of a combination of GABA agonists, baclofen and muscimol, attenuated the mean number of fentanyl infusions (n=30, 20 male, 10 female; p=0.001), supporting a role for this region in fentanyl reinforcement. This effect on intake was only apparent after more than 30 minutes of the session had elapsed (ANOVA time x treatment interaction, p=0.0089); this delayed effect was not due to GABA agonist diffusion, and appears related to differential impact on the load-up versus maintenance phase of self-administration behavior. To probe the role of CeA opioid receptors, we microinfused the opioid receptor antagonist, naltrexone, prior to self-administration sessions. Blockade of CeA opioid receptors by naltrexone dose-dependently increased intake (n=13, 5 male, 8 female; p=0.0257) and decreased median infusion latencies (p=0.0116), consistent with CeA opioid receptors being key mediators of fentanyl reinforcement. Sex did not interact with any of these effects. Taken together, these results demonstrate that the CeA is engaged by fentanyl self-administration when subjects have been trained under conditions that are not considered to induce strong dependence.

Disclosures: E. Chaloux-Pinette: None. **X. Tong:** None. **K. Ananth:** None. **V. Silva:** None. **P.H. Janak:** None.

Poster

652. Fentanyl Seeking

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Program #/Poster #: 652.11

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA037897
T32 DA028874

Title: Glp-1 receptor agonism in the interpeduncular nucleus decreases fentanyl reinstatement in male and female rats

Authors: *R. J. HERMAN, E. TYNER, S. ZEB, K. RAGNINI, H. D. SCHMIDT;
Univ. of Pennsylvania, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Fatal opioid overdose is a leading cause of preventable death in the United States, and over one half of all opioid overdose deaths are associated with synthetic opioids including fentanyl and its analogs. Uncovering the neural mechanisms of opioid seeking will facilitate the development of novel treatments for opioid use disorder that may help to decrease or prevent opioid overdose deaths. Our previous studies showed that systemic administration of the glucagon-like peptide-1 receptor (GLP-1R) agonist Exendin-4 (Ex-4) decreased intravenous fentanyl self-administration and the reinstatement of fentanyl-seeking behavior, an animal model of relapse, in rats. Given that GLP-1Rs are expressed throughout the brain, it is crucial to identify the GLP-1R-expressing circuits that mediate the efficacy of GLP-1R agonists in attenuating opioid seeking. GLP-1Rs are expressed at high levels in the interpeduncular nucleus (IPN), a brain region known to regulate the mesolimbic dopamine system. Based on our pilot studies with systemic Ex-4, we hypothesized that activation of GLP-1Rs in the IPN would attenuate fentanyl reinstatement. We trained male and female rats to self-administer intravenous fentanyl (1.25 μg/kg/infusion) for 21 days on a fixed-ratio 5 schedule of reinforcement. Drug taking was then extinguished by replacing the fentanyl solution with saline and turning off the contingent light cue. Once fentanyl taking was extinguished, reinstatement of opioid seeking was assessed following an acute priming injection of fentanyl and re-exposure to conditioned light cues. Prior to each reinstatement test, rats were pre-treated with intra-IPN infusions of vehicle or Ex-4 (0.01 or 0.1 μg). We showed that intra-IPN infusions of Ex-4 dose-dependently decreased drug- + cue-induced reinstatement of fentanyl seeking in male and female rats. Importantly, our preliminary data indicate that IPN Ex-4 inhibits fentanyl seeking at doses that do not affect body weight, chow intake, or pica. Additionally, we found that GLP-1Rs and mu opioid receptors are both expressed on IPN neurons that project to the LDTg, providing a potential mechanism for the suppressive effects of Ex-4 on opioid seeking. Further analysis revealed subregion-specific expression of GLP-1Rs on IPN GABA neurons that project to the LDTg. Overall, these results support a functional role of IPN GLP-1R activation in Ex-4's effect on opioid seeking and support the use of GLP-1R agonists as a potential treatment for fentanyl use disorder.

Disclosures: R.J. Herman: None. **E. Tyner:** None. **S. Zeb:** None. **K. Ragnini:** None. **H.D. Schmidt:** None.

Poster

652. Fentanyl Seeking

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Program #/Poster #: 652.12

Topic: G.09. Drugs of Abuse and Addiction

Title: Artificial light at night drives sex-specific increases in opioid reward-related behavior in mice

Authors: *D. D. BECKER-KRAIL, W. H. WALKER, II, J. R. BUMGARNER, R. J. NELSON;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: The ongoing opioid epidemic is the worst it has been in U.S. history. In 2021, the U.S. saw the highest annual drug overdose death toll ever recorded, with an estimated 107,000+ overdose deaths. Strikingly, nearly 75% of all overdose deaths involved opioid use - in particular, fentanyl accounted for 2/3 of them. Although a few therapeutic options exist to help manage symptoms and curtail use, there remains a dire need to understand the underlying biology and to discover novel therapeutic targets. Of particular interest, disrupted circadian rhythms have been associated with increased substance use and aberrant reward regulation. Moreover, with both the widespread adoption of modern lighting and the growing prevalence of night shift work, exposure to artificial light at night (ALAN) and subsequent circadian rhythm disruption (CRD) have become increasingly pervasive. Here we aimed to investigate the role ALAN-induced CRD may play in driving opioid reward-related behavior in mice. Male and female mice were first exposed to 4 weeks of either light days and dark nights (LD; 14 h of 150 lux:10 h of 0 lux) or light days and dim ALAN (14 h of 150 lux:10 h of 5 lux). Mice were then run through a two-bottle choice (2BC) task, whereby mice had access to a control quinine solution and a bottle containing either morphine (4 days: 0.4 mg/ml, 4 days: 0.7 mg/ml) or fentanyl (4 days: 1 μg/ml, 4 days: 10 μg/ml), in separate cohorts. In the morphine 2BC task, male mice exposed to ALAN (but not females) demonstrated a modest, but statistically significant increase in morphine consumption at the higher dose. This consumption significantly correlated with degree of CRD for males, but not for females. Notably, in the fentanyl 2BC task, female mice exposed to ALAN (but not males) demonstrated a striking increase in fentanyl consumption across both doses. Fentanyl consumption also significantly correlated with degree of CRD for females, but not for males. Together, these findings highlight a mechanism by which light at inappropriate times of day may increase propensity for opioid abuse. Future experiments will assess the effects of ALAN on opioid withdrawal, as well as use RNA-sequencing to investigate the potential molecular mechanisms underlying this differential phenotype.

Disclosures: D.D. Becker-Krail: None. **W.H. Walker:** None. **J.R. Bumgarner:** None. **R.J. Nelson:** None.

Poster

652. Fentanyl Seeking

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA050906
NIH DA051551
NIH DA018343
BBRF Young Investigator

Title: Microbiome knockdown in adolescence alters behavioral flexibility, fentanyl reinstatement, and protein expression in prelimbic cortex

Authors: *R. HOFFORD[1,3], A. L. SHIPMAN[3], W. WANG[4], J. J. CHOW[5], T. T. LAM[4], D. KIRALY[2,6];
[1]Wake Forest Sch. of Med., Winston-Salem, NC; [2]Wake Forest Sch. of Med., Winston-Salem, NY; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Yale Univ., New Haven, CT; [5]NIDA IRP, Baltimore, MD; [6]Icahn Sch. of Med., New York, NY

Abstract: Adolescence is the time of life when experimentation with substance use commonly begins. During this time many areas of the brain are undergoing drastic changes - most notably the prefrontal cortex (PFC). Given the importance of the PFC to drug reward, it is crucial to understand how environmental perturbations occurring during adolescence influence PFC function in the short and long-term. Previous work from our lab and others has demonstrated that a healthy gut microbiome is necessary for normal responses to drugs of abuse. However, little work has examined how microbiome disruption during adolescence will affect PFC-mediated behavior and PFC protein expression. In this series of studies, we examined the effect of microbiome knockdown on two PFC-dependent behavioral assays: behavioral flexibility and opioid reinstatement. To reduce the microbiome, rats in all experiments were given antibiotics (Abx) or water (H2O) for five days before the start of experiments. For experiment 1, adolescents and adults from H2O and Abx groups were trained to discriminate between two levers that differed in the probability of food pellet delivery - one lever delivering a reinforcer 25% of the time and the other delivering a reinforcer 75% of the time. After stable responding was achieved, the advantageous and disadvantageous levers were reversed to assess behavioral flexibility. Experiment 2 measured cue-induced reinstatement for fentanyl in H2O and Abx-treated adolescents. Additionally, the PFC from rats in experiment 2 was analyzed for global alterations in protein expression using unbiased mass spectrometry. As seen previously, microbiome knockdown produced robust effects on both behavior and protein expression. Adolescent rats with a reduced microbiome had more severe impairments in behavioral flexibility but exhibited lower rates of fentanyl-seeking, suggesting overall impairment in PFC function. In line with the behavior, abx rats exhibited a unique protein expression profile within PFC compared to H2O-treated rats. Future studies will attempt to link the alterations in protein expression to impaired PFC function induced by microbiome knockdown.

Disclosures: R. Hofford: None. **A.L. Shipman:** None. **W. Wang:** None. **J.J. Chow:** None. **T.T. Lam:** None. **D. Kiraly:** None.

Poster

652. Fentanyl Seeking

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

Title: Role of piriform cortex afferent projections in relapse to fentanyl seeking after food choice-induced voluntary abstinence

Authors: *S. M. CLAYPOOL, S. BEHDIN, J. ORIHUEL, Y. SHAHAM, D. J. REINER; IRP/NIDA/NIH, Baltimore, MD

Abstract: Background: We previously showed a role of piriform cortex (Pir) in relapse to fentanyl seeking after food choice-induced voluntary abstinence, a procedure that mimics abstinence due to availability of alternative non-drug rewards. Here, we used retrograde tracing to determine projection-specific activation of Pir afferent projections during fentanyl relapse by using Fos plus the retrograde tracer cholera toxin B (injected into Pir).
Methods: We trained male and female rats to self-administer palatable food pellets for 6 days (6-h/day) and fentanyl (2.5 microgram/kg/infusion, i.v.) for 12 days (6-h/day). We assessed relapse to fentanyl seeking after 14 voluntary abstinence days, achieved through a discrete choice procedure between fentanyl and palatable food (20 trials/day).
Results: Relapse to fentanyl seeking was associated with increased Fos expression in neurons in anterior insular (AI) and prelimbic (PL) cortex that project to Pir but not Pir-projecting cortical neurons in adjacent areas or Pir-projecting thalamic neurons. Preliminary anterograde tracing in AI or PL confirmed these AI-to-Pir and PL-to-Pir projections.
Conclusions: Results demonstrate a correlational role of AI-to-Pir and PL-to-Pir projections in relapse to fentanyl seeking after food choice-induced abstinence. In an ongoing experiment, we determine the causal role of these projections in relapse to fentanyl seeking after food choice-induced abstinence. We will present these results at the meeting.

Disclosures: S.M. Claypool: None. **S. Behdin:** None. **J. Orihuel:** None. **Y. Shaham:** None. **D.J. Reiner:** None.

Poster

652. Fentanyl Seeking

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 652.15

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5U01DA051373

Title: D-cysteine ethyl ester reverses fentanyl-mediated inhibition of “intrinsic Ca^{2+} activity” in neurons isolated from superior cervical ganglion

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Abstract: The opioid epidemic is a major health crisis in the U.S., resulting in an estimated 80,816 deaths in 2021, with an estimated 70% increase in emergency room visits for overdose treatment. Overdose results in Opioid-Induced Respiratory Depression (OIRD) which is treated by administration of competitive opioid receptor antagonists like naloxone. However, these drugs are not as effective against highly potent synthetic opioids, e.g. fentanyl, and can trigger severe withdrawal symptoms. There is an obvious unmet medical need for development of safe, effective, protective treatments that can alleviate OIRD without precipitating withdrawal while maintaining opioid-induced analgesic efficacy. The superior cervical ganglion (SCG) sends sympathetic input to the carotid body where the information is processed resulting in a compensatory increase in respiration. D-cysteine ethyl ester (D-CYSee) has been shown to prevent OIRD without affecting analgesia or inducing a withdrawal state in rats. Thus, we assessed the effects of D-CYSee on intrinsic calcium activity in heterogeneous cell cultures derived from the SCG of P0-P3 Sprague Dawley rat pups (N=21; 7 pups/culture). Cells were cultured for 12-days prior to loading with the fluorescent Ca^{2+} probe, Cal-520 AM, and imaged in "real-time" on an inverted microscope. We assessed changes in intrinsic unstimulated intracellular Ca^{2+} (iCa^{2+}) activity over 25-minutes in which cells were perfused under one of four treatments preceded by a control period: **1)** Ca^{2+} free, **2)** fentanyl (10,50,or 100nM), **3)** D-CYSee (1,10,or 100uM), and **4)** fentanyl + D-CYSee all conditions were followed by a drug washout. Under control conditions, cells displayed intrinsic Ca^{2+} activity at 1.2 ± 0.083 Hz. The activity was found to be dependent on the presence of extracellular Ca^{2+}. Administration of D-CYSee (1, 10, and 100uM) failed to induce any change in amplitude or frequency of intrinsic $_{i}Ca^{2+}$ activity ($p>0.05$). Administration of fentanyl at (10, 50, and 100nM) produced a dose-dependent inhibition of intrinsic iCa^{2+} activity ($p<0.05$) that was reversed during co-administration of D-CYSee (100uM)($p<0.05$). Washout of the drugs resulted in a prompt return to baseline intrinsic iCa^{2+} activity ($p>0.05$). This study provides the first evidence that OIRD may involve the shut-down of active SCG inputs to key sites such as the carotid bodies involved in respiratory control mechanisms. The ability of D-CYSee to effectively reverse the negative effects of fentanyl in the SCG may be a key mechanism by which D-CYSee overcomes OIRD. **Funding:** 5U01DA051373: Optimization of Novel Thiolesters as a Therapeutic Strategy for Combating Opioid Overdoses and Abuse

Disclosures: T.R. Nakashe: None. **Z.T. Knauss:** None. **A.C. Beard:** None. **S.J. Lewis:** None. **D.S. Damron:** None.

Poster

652. Fentanyl Seeking

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5U01DA051373:

Title: D-cysteine ethyl ester reverses fentanyl-mediated inhibition of intrinsic Ca^{2+}activity in neurons and astrocytes isolated from the prefrontal cortex

Authors: *Z. T. KNAUSS[1], M. R. SLUPEK[1], S. J. LEWIS[2], D. S. DAMRON[1];
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Abstract: The opioid epidemic is a major health crisis in the U.S., resulting in an estimated 80,816 deaths in 2021. Overdose results in Opioid-Induced Respiratory Depression (OIRD) which is treated with competitive opioid receptor antagonists such as naloxone. However, these drugs are not effective against highly potent synthetic opioids (i.e., fentanyl) and are poorly suited for the prevention and/or treatment of opioid-craving or addiction. D-Cysteine ethyl ester (D-CYSee) has been shown to prevent OIRD and disrupt the acquisition of fentanyl-induced seeking behaviors in rats. Activity levels in the prefrontal cortex (PFC) have been correlated in rats and humans with the formation and intensity of drug craving in response to drug-associated cues and contexts. Thus, we assessed the effects of D-CYSee and fentanyl on intrinsic calcium activity in heterogeneous cell cultures derived from the PFC of P0 Sprague Dawley rat pups (N = 12; 3 cultures, male = 6, female = 6). Cells were cultured for 12-days, loaded with a fluorescent Ca^{2+} probe, Cal-520 AM, and imaged on an inverted microscope. We assessed changes in intrinsic "unstimulated" intracellular Ca^{2+} ($_{i}Ca^{2+}$) activity over 25-minutes with perfusion under one of three treatment regiments preceded by a control period: **1)** fentanyl (10 nM), **2)** D-CYSee (10 uM), and **3)** fentanyl + D-CYSee all treatments were followed by a drug washout. Neurons and astrocytes were identified using live cell fluorescent probes NeuO and SR101. Under intervention free conditions, neurons (n_n = 169) and astrocytes (n_a = 562) displayed intrinsic Ca^{2+} activity at 0.29 ± 0.008 Hz and 0.19 ± 0.007 Hz respectively. A repeated measures ANOVA determined that mean frequency differed significantly between time points in both neurons ($F(2.70, 454.88) = 167.24$, $p < 0.001$) and astrocytes ($F(2.44, 1399.45) = 165.58$, $p < 0.001$). Post hoc analysis revealed that frequency significantly increased during the first 5 minutes of fentanyl treatment in neurons (-0.04 Hz, $p < 0.001$) and astrocytes (-0.03 Hz, $p < 0.001$), decreased below control during the last 10 minutes under continued fentanyl (0.10 Hz, $p < 0.001$), (0.09 Hz, $p < 0.001$) and failed to show a significant change under washout (0.004 Hz, $p = 1$) and (-0.016 Hz, $p = 0.043$). Co-administration of D-CYSee prevented a fentanyl-induced decrease in frequency during the last 10 minutes of fentanyl treatment (-0.014 Hz, $p = .373$) and upon treatment washout showed a significant increase in frequency over control (-0.07 Hz, $p < 0.001$). The ability of D-CYSee to effectively reverse the effects of fentanyl in the PFC may be a key mechanism by which D-CYSee prevents the acquisition of fentanyl-induced seeking behaviors and perhaps OIRD.

Disclosures: Z.T. Knauss: None. **M.R. Slupek:** None. **S.J. Lewis:** None. **D.S. Damron:** None.

Poster

652. Fentanyl Seeking

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Program #/Poster #: 652.17

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DAO38042-07

Title: D-cysteine ethyl ester disrupts acquisition of fentanyl seeking while preserving fentanyl-Induced motoric and analgesic efficacy

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Abstract: The opioid epidemic is a major health crisis in the U.S., resulting in an estimated 80,816 deaths in 2021 alone, with an estimated 70% increase in emergency room visits for overdose treatment. Overdose results in Opioid-Induced Respiratory Depression (OIRD) which is treated by administration of competitive opioid receptor antagonists such as naloxone. However, these drugs are not as effective against highly potent synthetic opioids, particularly fentanyl, and can trigger severe withdrawal symptoms e.g., anxiety, nausea, and vomiting. Recently, D-Cysteine ethyl ester (D-CYSee) has been shown to prevent OIRD without affecting analgesia or inducing a withdrawal state in rats. Thus, we assessed the effects of D-CYSee administration on the acquisition of fentanyl-induced seeking behaviors, anxiety, and locomotion using a rat model of conditioned place preference (CPP) and open field testing (OFT). Long Evans rats (N = 60; male = 30, female = 30) underwent place conditioning in a three-chamber apparatus for eight days under: **1)** saline (1 ml/kg, i.p.) - fentanyl (male 5 ug/kg / female 50ug/kg, s.c.), **2)** D-CYSee (10 mg/kg, i.p.) - saline, **3)** D-CYSee (100 mg/kg, i.p.) - saline, **4)** D-CYSee (10 mg/kg) - fentanyl, or **5)** D-CYSee (100 mg/kg) - fentanyl. Extinction testing was conducted for seven days or until extinction criteria were met. Next Long Evans rats (Male, N = 24) underwent a single 5-minute OFT for anxiety under: **1)** saline - saline, **2)** saline - fentanyl, **3)** D-CYSee (100 mg/kg, i.p.) - saline, or **4)** D-CYSee - fentanyl. We found that 5 ug/kg fentanyl in males and 50 ug/kg in females induced a significant increase in the percent time spent in the paired chamber by 135 ± 6% ($p < 0.0001$) and 90.5 ± 37% ($p<0.005$) respectively, over saline control. 10 mg/kg D-CYSee significantly reduced the percent time spent in the paired chamber compared to fentanyl controls -90.4 ± 29% ($p < 0.05$) in females, but failed to induce a significant change in males, though a negative trend -13.5 ± 5.0% ($p > 0.05$) was observed. Pretreatment with 100 mg/kg D-CYSee significantly reduced the percent time in the paired chamber compared to fentanyl controls -60.1 ± 4.4% ($p < 0.0001$) in males and -97.7 ± 28.8% ($p < 0.01$) in females. D-CYSee alone failed to induce a significant CPP/CPA compared to control. Further, open field testing revealed that D-CYSee failed produce maladaptive responding and that fentanyl induced immobility was preserved. Thus, D-CYSee disrupted the acquisition of fentanyl seeking in a sex and dosage specific manor without altering motoric or analgesic effects of fentanyl suggesting that D-CYSee has therapeutic potential to reduce addiction vulnerability during prescribed opioid use.

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Poster

652. Fentanyl Seeking

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Title: Pain and sex-specific effects on mesolimbic circuitry during fentanyl self-administration

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Abstract: Over half of US adults report having pain. Opioids are potent analgesics commonly prescribed for pain but are highly prone to abuse. Clinical evidence suggests that men are more vulnerable to negative outcomes associated with opioid use than women, which may be exacerbated by pain. Supporting this, we recently found that inflammatory pain increases fentanyl intake over time selectively in male rats, but the precise mechanisms underlying this phenomenon remain unclear. Opioid reward processing and motivated behavior is driven by mesolimbic dopamine release from the ventral tegmental area (VTA) in the nucleus accumbens (NAc). Hence, we tested the hypothesis that pain increases fentanyl intake in males to due to sex and pain-specific increases in fentanyl-evoked activity of VTA dopamine neurons projecting to the NAc. To do this, male and female TH-Cre rats (2-3 m.o.) were injected with a Cre-dependent GCaMP in the VTA and an optic fiber was implanted in the shell of the NAc. Rats were implanted with an IV catheter and subjected to pain (hind paw injection of Complete Freuds Adjuvant) or no pain (saline) prior to undergoing fentanyl self-administration. Correct lever presses during 15 daily 2-hour sessions were reinforced with illumination of a cue light and a fentanyl infusion of 5µg/kg or 2µg/kg (IV). To simultaneously permit self-administration and detect calcium transients from dopaminergic terminals in the NAc from the VTA, we used wireless in vivo fiber photometry. Photometry recordings occured throughout the training sessions at least every 5 days and DF/F signals were aligned with fentanyl-reinforced lever responses. Mechanical sensitivity was measured on every other day of training to confirm the presence of pain. Time-dependent effects on VTA to NAc calcium transient activity varied as a function of sex, pain, and time. In particular, fentanyl-evoked DF/F signals from male rats with pain increased in amplitude after 10 days of fentanyl self-administration, a timepoint when increases in fentanyl consumption were previously observed. Finally, to tease apart drug- and cue-specific effects on VTA to NAc activity, we assessed lever responses and photometry signals during subsequent extinction training, cue-induced reinstatement, and fentanyl infusions in the

absence of cues. We identified distinct impacts of fentanyl and fentanyl-paired cues on VTA to NAc GCaMP fluorescence, suggesting this circuit encodes discrete components of opioid reinforcement. Together, these findings demonstrate a sex-specific influence of pain on opioid use and mesolimbic dopamine function in a circuit- and cell-type-specific manner.

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Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Support: NIH/NIAAA AA006420
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NIH/NIAAA AA028549
NIH/NIAAA T32 AA007456

Title: ADX106772, an mGlu2 receptor positive allosteric modulator, selectively attenuates oxycodone- but not sweetened condensed milk-seeking behavior

Authors: J. M. ILLENBERGER[1], F. J. FLORES-RAMIREZ[1], A. MATZEU[1], R. LUTJENS[2], *R. MARTIN-FARDON[1];
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Abstract: Opioid abuse and overdose have risen to epidemic proportions in the United States. Prescription opioids, such as oxycodone, are potent analgesics that are used to treat and manage pain. Oxycodone is the most commonly abused prescription drug. Treatments for opioid use disorder (OUD) aim to reduce vulnerability to relapse by reducing sources of reinforcement to seek drug (i.e., reducing acute drug effects or drug withdrawal/craving). Because drugs of abuse elicit glutamate release acutely, interest has grown in targeting the glutamatergic system to reduce drug taking and seeking. This study tested whether ADX106772, a positive allosteric modulator of the metabotropic glutamate 2 receptor (mGluR2), would reduce oxycodone intake and conditioned reinstatement while having less or no effect on the consumption or motivation to seek a palatable conventional reinforcer (i.e., sweetened condensed milk; SCM). Male Wistar rats were trained to self-administer oxycodone (0.15 mg/kg/infusion, i.v.,12 h/day) or SCM (diluted 2:1 v/v in H2O, 30 min/day) for 13 days, in the presence of a contextual/discriminative stimulus (S^D). Doses of ADX106772 (0-10 mg/kg, s.c.) were then administered in a within-subjects Latin-square design 30 mins prior to self-administration sessions. The rats then underwent 2-h daily extinction training, during which oxycodone, SCM and the S^D were withheld. After extinction, the ability of ADX106772 to prevent S^D- induced conditioned reinstatement of oxycodone- or SCM-seeking behavior was tested. The rats self-administering oxycodone progressively escalated their intake over the 13 days of training and exhibited

significant physical signs of opioid withdrawal confirming dependence. At 1, 3, and 10 mg/kg, ADX106772 significantly reduced oxycodone self-administration ($F(4,150)= 4.31$, $p\leq 0.05$) and conditioned reinstatement ($F(5,180)=2.57$, $p \leq 0.05$) while having no effect on SCM administration or reinstatement. ADX106772 reduced oxycodone taking and seeking and showed a lack of effect on the motivation to consume and seek a palatable conventional reinforcer, supporting the mGluR2 positive allosteric modulation approach as a potential treatment of prescription OUD. ADX106772 was supplied by Addex Therapeutics.

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Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

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AA028549
T32 AA007456

Title: Blockade of orexin receptors in the posterior paraventricular nucleus of the thalamus with the dual orexin receptor antagonist, Suvorexant, selectively prevents stress-induced reinstatement of oxycodone-seeking behavior in male rats

Authors: *J. M. ILLENBERGER, F. FLORES-RAMIREZ, G. PASCASIO, R. MARTIN-FARDON;
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Abstract: Oxycodone is a potent analgesic used to treat and manage pain and is one of the most commonly abused prescription drugs. The orexin (Orx) system is recruited by drugs of abuse, and it has been demonstrated that blockade of Orx receptors (OrxR) can prevent oxycodone, cocaine, and alcohol-seeking behavior. Because Orx transmission in the posterior paraventricular nucleus of the thalamus (pPVT) is pivotal in mediating drug-seeking behavior, the present study tested whether intra-pPVT administration of the FDA-approved dual orexin receptor antagonist, suvorexant (Sx), could prevent foot shock stress-induced reinstatement of oxycodone-seeking behavior. Seventeen Male Wistar rats were trained to self-administer oxycodone (0.15 mg/kg/infusion, i.v.) by voluntary lever pressing, 8 h/day for 21 days (FR1, TO20). A separate group of rats was trained to orally self-administer a highly palatable food reward (sweetened condensed milk, [SCM], dilution 2:1 v/v, FR1, TO20). After training, rats underwent 2-h daily extinction training, during which oxycodone and SCM were withheld. Following extinction, the effect of intra-pPVT Sx (15 µg/0.5 µl) on stress-induced reinstatement of oxycodone and SCM-

seeking behavior was tested and assessed with regression methods. Rats progressively escalated oxycodone self-administration ($F(1,19)= 4.9$, $p < 0.05$) with training and manifested significant physical signs of opioid withdrawal confirming dependence. Footshock stress significantly reinstated oxycodone-seeking behavior, and intra-pPVT administration of Sx blocked this effect ($F(1,4)= 7.8$, $p < 0.05$). For SCM, rats readily acquired self-administration ($F(1,19)= 44.4$, $p < 0.001$), and stress significantly reinstated SCM-seeking behavior. However, in contrast to what was observed for oxycodone, intra-pPVT Sx did not modify stress-induced reinstatement of SCM-seeking behavior ($p > 0.05$). The present findings suggest that OrxR signaling within the pPVT contributes selectively to stress-induced reinstatement of oxycodone-seeking behavior and may provide a promising target for future therapeutics to prevent stress-induced craving and relapse to prescription opioids. This work was supported by The National Institute on Alcohol Abuse and Alcoholism (grant no. AA006420, AA026999, AA028549, and T32 AA007456).

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Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Spanish Ministerio de Sanidad Grant “ESF Investing in your future”, Plan Nacional Sobre Drogas 2021I070

Title: (S)-ketamine decreases mu opioid receptors and increases heroin self-administration: implications for abuse liability

Authors: *M. R. LEVINSTEIN[1], M. L. CARLTON[1], T. DI IANNI[3], E. N. VENTRIGLIA[1], A. RIZZO[4,5], J. L. GOMEZ[1], R. C. BUDINICH[1], Y. SHAHAM[2], R. AIRAN[3], C. A. ZARATE, Jr.[6], J. BONAVENTURA[4,5], M. MICHAELIDES[1,7];
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Abstract: While widely regarded as a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, (S)-ketamine also binds to and activates the mu opioid receptor (MOR). However, the contribution of MORs to (S)-ketamine's *in vivo* pharmacological actions and abuse liability is not well understood. Using rats, we show that naltrexone pretreatment significantly decreased intravenous (IV) self-administration of (S)-ketamine without affecting self-administration of food. IV (S)-ketamine, at a dose equivalent to a single self-administration session, significantly decreased binding of [^{18}F]fluoroethyl-diprenorphine (FE-DPN), a radioligand which preferentially binds to MORs *in vivo*, in the medial prefrontal cortex, and thalamus. Functional ultrasound imaging revealed that a single IV (S)-ketamine injection, at the self-administered unit dose, increased activity in the nucleus accumbens and naltrexone pretreatment significantly decreased this response. 8 days of exposure to IV (S)-ketamine led to significant decreases in [^{18}F]FE-DPN binding in several MOR-rich brain regions, which were confirmed postmortem as reductions in MOR density and function. Additionally, IV (S)-ketamine exposure for 8 days significantly increased subsequent heroin IV self-administration and intake. Our results show that IV self-administration of (S)-ketamine in rats is mediated by MORs and that repeated (S)-ketamine exposure produces MOR desensitization and increased heroin seeking.

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Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Title: 6-O-(2-[^{18}F]Fluoroethyl)-6-O-desmethyl-diprenorphine ([^{18}F]FE-DPN) preferentially binds to mu opioid receptors *in vivo*

Authors: *R. C. BUDINICH[1], M. R. LEVINSTEIN[1], E. N. VENTRIGLIA[1], J. L. GOMEZ[1], J. MARTON[2], G. HENRIKSEN[3,5,4], D. HOLT[6], R. F. DANNALS[6], M. G. POMPER[6], C. A. ZARATE, Jr.[8], J. BONAVENTURA[9,10], M. MICHAELIDES[1,7];
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Abstract: Diprenorphine (DPN) and 6-O-(2-[^{18}F]Fluoroethyl)-6-O-desmethyl-diprenorphine ([^{18}F]FE-DPN) are considered to be non-selective opioid receptor ligands due to their similar *in vitro* affinity at the mu (MOR), kappa (KOR), and delta (DOR) opioid receptors. However, the *in vivo* selectivity of these compounds is not well understood. Here we characterize [^{18}F]FE-DPN synthesized from the novel precursor, 6-O-(2-tosyloxyethoxy)-6-O-desmethyl-3-O-trityl-diprenorphine (TE-TDDPN), using a one-pot, two-step nucleophilic radiosynthesis to image opioid receptors in rats and mice using positron emission tomography (PET). Then, utilizing MOR (Oprm1) knockout (KO) mice, we examined binding selectivity of [^{18}F]FE-DPN using PET and [^{3}H]DPN using *ex vivo* and *in vitro* autoradiography. In rats (n=6), [^{18}F]FE-DPN [~22.2 Megabecquerel (MBq), intravenous (IV)] accumulated in regions with high opioid receptor density (thalamus, periaqueductal grey, hypothalamus, striatum, superior colliculus, and brainstem) and reached a steady state at ~50 minutes post injection. This accumulation was fully blocked by pretreatment with the opioid antagonist naltrexone [10mg/kg, subcutaneous (SC)]. In mice, [^{18}F]FE-DPN (~7.4MBq, SC) had high accumulation in the brains of wildtype (WT) animals (n=6), but did not accumulate in the brains of MOR KO mice (n=6), where binding had negligible difference from background. We then injected [^{3}H]DPN in MOR KO (n=3) and WT (n=3) mice to assess [3H]DPN brain uptake and found negligible brain uptake of [^{3}H]DPN in MOR KO mice. Naltrexone pretreatment nearly abolished [^{3}H]DPN uptake in WT mice and had no significant effect on binding in MOR KO mice. These data indicate that whereas FE-DPN and DPN are non-selective opioid receptor ligands *in vitro*, they preferentially bind to MOR *in vivo*. Additionally, this is a demonstration that *in vitro* ligand engagement findings are not necessarily translatable to what may occur under *in vivo* conditions.

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PTSD., Has assigned patent rights to the U.S. government but will share a percentage of any royalties that may be received by the government.. **J. Bonaventura:** None. **M. Michaelides:** 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.; Received research funding from AstraZeneca, Redpin Therapeutics, and Attune Neurosciences.

Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Program #/Poster #: 653.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA047265
NIDA T32 DA007237

Title: Social isolation stress influences oxycodone self-administration in mice in a sex-specific manner

Authors: *E. M. BLACK[1], M. C. KNOUSE[1], E. A. BIRMINGHAM[1], I. L. TODOROVSKI[1], L. A. BRIAND[2];
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Abstract: Adolescence is a crucial period for social development. As such, isolation during adolescence can lead to socialization and stress-related problems during adulthood. Additionally, adolescent social isolation is a stressor that influences the propensity to develop substance use disorders later in life. Our lab has previously demonstrated that adolescent social isolation stress increases cocaine-seeking and motivation to self-administer cocaine in male and female mice. In contrast, previous studies suggest that other forms of early life stress may confer resistance to opioid seeking. Therefore, the current study aimed to determine if social isolation stress leads to increased or decreased opioid self-administration. Specifically, we examined the impact of adolescent social isolation stress on oxycodone self-administration in male and female mice. Consistent with previous results, adolescent-onset social isolation did not alter the ability of mice to learn operant self-administration for food. However, in contrast to the previous increases in cocaine seeking, adolescent social isolation stress led to a decrease in oxycodone self-administration in females, while not altering oxycodone self-administration in males. Additionally, preliminary data indicate that social isolation stress may increase motivation to self-administer oxycodone on a progressive ratio (PR) schedule of reinforcement in males, while not altering PR responding in females. Taken together, these data suggest sex specific effects of adolescent social isolation stress on both oxycodone taking and motivation for oxycodone. Given we have seen effects on fixed ratio (FR1) responding in females but not PR responding, it is possible that the decrease in oxycodone self-administration is due to an increased sensitivity to the drug rather than a decrease in the reinforcing efficacy. Ongoing studies are examining a dose

response curve for both the reinforcing effects and the conditioned rewarding effects of oxycodone. Results from the present studies suggest that exposure to social isolation stress during adolescence leads to sex-specific alterations in oxycodone taking and seeking.

Disclosures: **E.M. Black:** None. **M.C. Knouse:** None. **E.A. Birmingham:** None. **I.L. Todorovski:** None. **L.A. Briand:** None.

Poster

653. Opioids: Pharmacology and Circuit Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 653.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA049837
NIDA R01 R01 DA047265
NIH T32 T32 DA007237

Title: The role of PKMζ in oxycodone self-administration and accumbal electrophysiology

Authors: *M. C. KNOUSE[1], T. HOUSER[4], L. BIRMINGHAM[2], L. A. BRIAND[3];
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[4]Psychology, Univ. of Oregon, Eugene, OR

Abstract: The protein kinase C (PKC) family works to control the function of other proteins via phosphorylation. One member of this kinase family, PKMζ, is constitutively active and found exclusively in the central nervous system. While predominantly studied for a potential role in learning and memory, PKMζ also plays a role in drug use. Constitutive deletion of PKMζ potentiates cocaine self-administration and cue-induced reinstatement in both male and female mice. Additionally, disrupting PKMζ leads to increased ethanol consumption. However, the role of PKMζ in opiate reward is unknown. The current experiments examined the effect of constitutive PKMζ deletion on oxycodone self-administration in male and female mice. We found that PKMζ knockout significantly potentiates oxycodone self-administration in both sexes. We next went on to examine the influence of PKMζ knockout on synaptic plasticity in the nucleus accumbens in both naïve and oxycodone-experienced animals. We found a significant effect of PKMζ knockout on short-term plasticity following oxycodone experience, as evidenced by alterations to paired pulse ratios. Paired pulse ratios in PKMζ knockout mice are significantly increased following oxycodone self-administration, an effect that is present in both sexes. This indicates PKMζ deletion alters presynaptic plasticity following oxycodone-experience in both sexes. Experiments are ongoing to examine the role of PKMζ in motivation to acquire opioids and whether these effects are maintained at lower doses of oxycodone. Together, current results suggest that PKMζ plays a role in opioid drug-taking and modulates subsequent synaptic plasticity in the nucleus accumbens.

Disclosures: **M.C. Knouse:** None. **T. Houser:** None. **L. Birmingham:** None. **L.A. Briand:** None.

Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Program #/Poster #: 653.07

Topic: G.09. Drugs of Abuse and Addiction

Support: DA051972

Title: Rat strain differences in opioid addiction-like behaviors

Authors: *R. QIAO[1], A. MARTINEZ[1], S. DIRIK[1], C. CROOK[1], C. BENNER[2], F. TELESE[2], G. DE GUGLIELMO[1];
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Abstract: In the past two decades, a sharp increase in the prescription of opioid medications to treat acute and chronic pain has led to the misuse of both prescription and illegal opioids. This contributed to a national epidemic that has devastating consequences on public health as well as social and economic welfare. Thus, there is an urgent need to develop better treatments for opiate addiction, which requires a better understanding of its biological basis. Genetic variation is one of the most important factors that may contribute to individual differences in susceptibility to opioid use disorder (OUD), which also has huge implications for clinical practices. Here, we performed a behavioral screening in four inbred rat strains (ACI/N, BN/SsN, WKY/N, and F344/N) to identify strain differences in motivation to seek oxycodone during abstinence and other behavioral traits relevant to OUD. We used extended access to intravenous oxycodone self-administration (12 h/day, 150 mg/kg/inj) as a model of OUD. We used a fixed ratio schedule of reinforcement to measure the escalation of oxycodone self-administration and a progressive ratio schedule of reinforcement to measure the motivation in drug-taking. Moreover, we included additional behavioral assays to measure traits associated with opioid use in humans, including tolerance to the analgesic effects of oxycodone (tail immersion test), withdrawal-induced hyperalgesia (von Frey test), and oxycodone-induced respiratory depression (pulse oximeter). Finally, we measured oxycodone seeking during protracted abstinence (4 weeks) by re-exposing the animals to the same environment and cues previously associated with oxycodone self-administration. The results showed strain differences in most of the measures including oxycodone metabolism. Interestingly, BN/NHsd and F344/N had similar drug intake and metabolism but showed significantly different behaviors in response to opioids. Overall, these findings offer a foundation for the identification of genetic and molecular variants that underlie different aspects of the opioid addiction process not related to metabolism or intake.

Disclosures: **R. Qiao:** None. **A. Martinez:** None. **S. Dirik:** None. **C. Crook:** None. **C. Benner:** None. **F. Telese:** None. **G. de Guglielmo:** None.

Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Program #/Poster #: 653.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant K99DA054265
NIDA Grant R01DA044315
NIDA Grant F31DA053724

Title: Dissecting opioid-sensitivity of ventral tegmental area dopamine subpopulations

Authors: *B. JUAREZ[1], M. LOVELESS[1], M. JOHNSON[1], D. BAN[1], J. ELUM[2], M. QUINLAN[1], L. S. ZWEIFEL[1];
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Abstract: Ventral tegmental area (VTA) dopamine neurons and their projections to the nucleus accumbens (NAc) are critical for associative learning, motivation, and reward processing. Dysregulation of VTA dopamine neurons are thought to contribute to the progression of substance-use disorders, including opioid-use disorder. Canonically, acute exposure to opioids, including morphine, increases VTA dopamine activity and dopamine release in the NAc via inhibition of GABAergic inputs that express mu-opioid receptors (MORs), which synapse onto VTA dopamine neurons. The functional diversity of VTA dopamine subpopulations and their differential contributions to healthy and disordered behavioral processes is becoming increasingly appreciated. The Zweifel lab discovered a method to genetically isolate subpopulations of VTA dopamine neurons that project to subregions of the NAc. Corticotrophin releasing hormone receptor 1-Cre VTA (*Crhr1*$_{VTA}$) dopamine neurons project to the NAc Core and impact cue-food associations. Cholecystokinin-Cre VTA (*Cck*$_{VTA}$) dopamine neurons project to the NAc Shell and impact motivation and performance of conditioned food-seeking behaviors. Yet, whether opioids, such as morphine, impact regulation of specific subpopulations of VTA dopamine neurons to morphine regulate associative learning uniformly or differentially are not well resolved. Here, we implemented the genetic strategy with histological, electrophysiological and photometric approaches to determine how morphine regulates VTA dopamine subpopulations. We found differences in baseline intrinsic properties of these subpopulations and differences in responsivity to morphine exposure. We have also profiled the calcium activity of these subpopulations throughout morphine conditioned place preferences for these subpopulations.

Disclosures: B. Juarez: None. **M. Loveless:** None. **M. johnson:** None. **D. Ban:** None. **J. Elum:** None. **M. Quinlan:** None. **L.S. Zweifel:** None.

Poster

653. Opioids: Pharmacology and Circuit Mechanisms

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Program #/Poster #: 653.09

Topic: G.09. Drugs of Abuse and Addiction

Support: ACADIA Pharmaceuticals grant
U of Houston-Clear Lake BioBehavioral Research Fund

Title: A 5-HT_{2A}inverse agonist attenuates morphine withdrawal syndrome in the rat.

Authors: *D. H. MALIN[1], P. TSAI[1], E. R. MORALES[1], J. R. NGUYEN[1], S. A. NIÑO[1], K. S. SADHEORA[1], C. P. WARD[1], E. S. BURSTEIN[2], G. L. MORENO[1];
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Abstract: An earlier study found that pimavanserin, a 5-HT_{2A} receptor inverse agonist in current medical use, attenuated the nicotine withdrawal syndrome. Given relationships between nicotine and opiate physical dependence, it was hypothesized that pimavanserin would attenuate morphine withdrawal syndrome in the rat. Twenty-seven rats were rendered morphine-dependent by seven days of continuous 0.6 mg/kg/hr morphine sulfate. On the seventh day, morphine infusion was terminated and a day later, rats were injected with 0.3 or 1.0 mg/kg pimavanserin or saline alone (a positive control for untreated morphine dependence). A non-dependent negative control group of ten rats were infused with saline alone and injected with saline. One hour after injections, all rats were observed under blind conditions for somatically expressed behavioral withdrawal signs. Compared with morphine-dependent, saline-injected rats, the non-dependent rats and both morphine-dependent pimavanserin dose groups all had significantly reduced overall withdrawal signs, p < 0.001, based on Tukey's HSD test for non-independent pairwise comparisons. The effect of morphine infusion on subsequent withdrawal signs was totally reversed by the higher pimavanserin dose and almost entirely reversed by the lower dose. Compared to the dependent, saline-injected group, the predominant withdrawal sign, wet dog shakes, was significantly, p < 0.001, reduced by both pimavanserin dose groups. The second most frequent withdrawal sign, abdominal writhes, were also significantly, p < 0.01, reduced by both pimavanserin dose groups. There were large effect sizes for all of these comparisons. Since pimavanserin is highly selective for the 5-HT_{2A} serotonin receptor, the results suggest that activation of this receptor contributes to opiate physical dependence, and that 5-HT_{2A} inverse agonists may powerfully reduce opiate dependence and withdrawal syndrome.

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Poster

653. Opioids: Pharmacology and Circuit Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 653.10

Topic: G.09. Drugs of Abuse and Addiction

Title: Multiple Toxic Adulterants Now Found in Illicit Fentanyl

Authors: S. KAUSHIK[1], D. M. MARTIN[1], W. LI[1], T. BROWNE[2], *M. S. GOLD[3];
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[3]Washington Univ., St. Louis, MO

Abstract: ***Background:*** Street drug use is now a leading public health concern, major cause of death of Americans under the age of 30, and fentanyl overdoses have reached an all-time high. In 2021 alone, 11,201 pounds of fentanyl were seized by the Drug Enforcement Agency (DEA), which has the potential to kill every person in the USA seven times over. Our research objective was to determine if fentanyl alone was present in street drugs or if there were other drugs that, in combination, could be responsible for these deaths. In 2021, our group in collaboration with the U.S. State Department and Colombo Plan studied the composition of street drugs in the United States with a focus on increased risk of Covid-19 infection. The results showed a dramatic increase in the amount of street drugs that are combined with toxic adulterants. These generally refer to other street drugs, ethical and veterinary pharmaceuticals, drug manufacturing byproducts and other chemicals added to the street drugs to enhance their effects and increase profits. This report is a retrospective analysis of these data with a focus on fentanyl to note if toxic adulterants are added to this street drug.***Methods:*** We selected data from Ohio as it has been a center of fentanyl use. 189 samples of street drugs seized by law enforcement were tested for a wide range of street drugs and toxic adulterants using Quadrupole Time of Flight (QTOF) mass spectrometry, a technology that is not routinely found in law enforcement laboratories. The QTOF analysis was performed by a private laboratory with forensic certification used by the FBI and DEA, and it screened for approximately 100 street drugs and toxic adulterants.***Results:*** Samples with fentanyl as the dominant drug were grouped together. This included 86 samples or 45.5% of the total sample cohort. Of these, 69 fentanyl samples (80.2%) had at least one (1) toxic adulterant and ranged up to 18 toxic adulterants in a single sample. Only 17 of the samples (19.8%) were fentanyl alone. The most common toxic adulterant was lidocaine (29 samples), followed by diphenhydramine (26 samples), cocaine and tramadol (25 samples each) and heroin (24 samples). These toxic adulterants often were seen together in a single fentanyl sample. It is important to note that fentanyl was the most abundant drug in these samples.***Conclusion:*** While fentanyl use alone poses a severe health risk, the addition of up to 18 toxic adulterants in a single fentanyl sample is alarming. Also, the synergistic effects on the human brain and body are unknown. These new combinations may be partially responsible for recent increasing death rates due to the negative side effects of multiple toxic adulterants added to fentanyl.

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Poster

654. Working Memory

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Program #/Poster #: 654.01

Topic: H.05. Working Memory

Support: SIP-20221404
COFAA-Fellowships
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Title: Systemic and intra-reticular thalamic administration of haloperidol induces short-memory disfunction in the rat

Authors: H. GUTIÉRREZ-GUERRERO, C. EVANGELISTA-ARZATE, K. BARÓN-QUIROZ, M. GARCIA-RAMIREZ, *E. C. CHUC-MEZA;
Natl. Sch. Biolog Sci. IPN, Natl. Sch. Biolog Sci. IPN, Tlalnepantla De Baz, Mexico

Abstract: Anterior and mediodorsal thalamic nuclei have been linked to memory and learning disfunctions (Aggleton & Nelson, 2015; Cross et al. 2012; Pernaudeau et al. 2018). Nevertheless, role in memory of thalamic reticular nucleus (TRN), a key thalamic structure to control the information interchange between thalamus and cortex, has scarcely been studied. TRN has a common dopaminergic innervation from *substantia nigra compacta* with *globus pallidus* and striatum named as extra-striatal innervation (Anaya-Martinez et al. 2006). Functional relevance of this innervation has been evaluated by local 6-hydroxidopamine lesions in TRN and *globus pallidus* producing anxiety and memory alterations, respectively (Picazo et al. 2009; Baron-Quiroz et al. 2021). So, in this work the effect of acute dopaminergic blocking by systemic and direct TRN administrations of haloperidol on short memory was assessed. All animal procedures were performed in accordance with national and international guidelines for care and use of laboratory animals (NOM-062-ZOO-199 & NIH Guide). Adult male Wistar rats were used (n=6-8/group) to test their performance on novel object recognition task (NORT) and locomotor activity. In NORT short-memory was evaluated measuring the exploration time of a novel object and another previously known and using these times to calculate a new object recognition index (NORI). First the systemic effect of haloperidol was evaluated at doses of 0.04, 0.07, 0.1 and 0.7 mg/kg observing a reduction of NORI at 0.1 and 0.7 mg/kg. Only 0.7 mg/kg affected locomotor activity. Subsequently intra-cerebral cannulas directed to TRN were implanted in the proper stereotaxic coordinates calculated using Paxinos & Watson rat brain atlas (2014). After surgical recuperation period the unilateral injection of 200 μM of haloperidol elicited a statistically significant reduction on NORI without effects on locomotor activity. These results shows that dopaminergic D2 block in TRN reduces short-memory in recognition tasks implying that dopaminergic activity of extra-striatal way to this nucleus and *globus pallidus* is needed for a correct performance in recognition memory. Finally, it could be possible that damage to this innervation it would be related to the cognitive disfunction showed by Parkinson patients in early stage.

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Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.02

Topic: H.05. Working Memory

Title: Effects of consumption of a perinatal Western diet in combination with aerobic exercise on cognitive development and early plasticity of CD-1 mouse progeny

Authors: *S. ROSALES-HERRERA[1], R. PEDRAZA-MEDINA[3], J. GUZMÁN MUÑIZ[4], N. A. MOY-LOPEZ[5], O. GONZALEZ-PEREZ[6], M. ORTIZ-VALLADARES[2];
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Abstract: Pregnancy demands physical and behavioral changes to respond to the needs of this period. The western diet (WD) combined with the lack of physical activity during pregnancy can alter the development of offspring due to alterations in the cytogenetic processes necessary for the consolidation of the Central Nervous System (CNS). Physical exercise (PE) has cognitive and physiological benefits, which can be an effective alternative to counteract the damage to offspring caused by WD consumption during pregnancy. This study aims to identify the effects of perinatal WD and PE on early cognitive plasticity in CD1 mouse pups. Adult females (n=8) were exposed to PE for one hour for 6 weeks (4 before and 2 during gestation). Another group of females (n=8) did not perform any physical activity in that period. At beginning of gestation, 4 exercised mothers were fed with the WD, like another 4 without activity. The remaining 8 mothers had a balanced diet (DB) throughout the process and pups were weaned at postnatal day 21 (PD21). Pups were cognitively assessed with the object recognition test (OR) from P21-PD23 and the T-maze with the spontaneous alternation (LT) assessment from PD24-P26. The analysis result showed that the gestational consumption of WD had effects on the physical development of the offspring, with the offspring of mothers with WD+PE having more weight, the results were equally significant, in brain weight ($p<0.05$). Regarding, learning and spatial memory, the offspring of WD-PE and WD+PE mothers were less efficient in the OR test, exploring the novel object for less time at 30 minutes and 24 hours ($p<0.05$). Finally, the groups of WD mothers had a lower number of spontaneous alternations during the second trial compared to the offspring of BD+EF mothers. The combination of adequate nutrition and physical activity during pregnancy are protective factors for the healthy neurodevelopment of offspring and precursors of cognitive plasticity. Understanding the early neurobiological effects of WD during gestation is a means to pursue studies based on the brain mechanisms involved in nutrition and physical activities during vulnerable periods as gestation.

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Poster

654. Working Memory

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Topic: H.05. Working Memory

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Title: An alteration of serial dependence in an aging population with mild cognitive impairment

Authors: *C. POUNGTUBTIM[1], K. BENJASUPAWAN[1], P. SOOKPRAO[2,1], S. ITTHIPURIPAT[2], C. CHUNHARAS[1,3];
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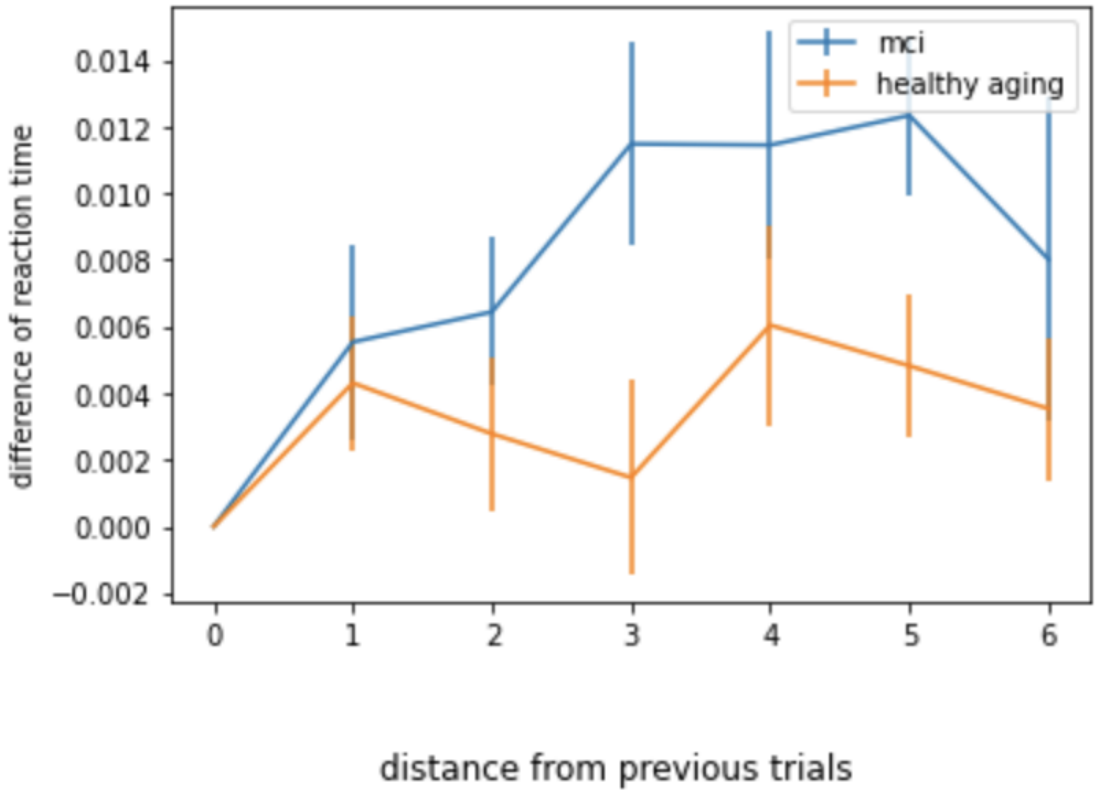
Abstract: In an ever changing world, retention of past information is necessary to stabilize our visual perception. This adaptive process is called serial dependence. While an effect was evidently found in several studies in adults, little is known about an effect of serial dependence in a healthy aging population or an aging population with mild cognitive impairment (MCI). Two possible theories can be seen in MCI patients. First, retention of previous memory could be difficult in MCI patients due to memory impairment. On the other hand, an effect of serial dependence might be stronger. As memory impairment could cause the patients to rely more on previous information. To test our hypotheses, our group recruited 17 healthy aging adults and 19 MCI patients aged from 55 to 82 years old. Visual search task was used to evaluate the subject's attention and working memory. All 12 stimuli were spatially arranged in a circular fashion. The cue appears on the screen to make participants attend to the specific location. Then, participants have to answer whether the shape at the target location is a diamond shape or hourglass. Accuracy and reaction time were recorded. We found that when the current stimulus appears in the same position as the previous trial. MCI patients responded faster and more accurately than when the stimuli appeared in other positions. (p-value = 0.0002 and 0.006 respectively) While in a healthy aging group, there was no difference between accuracy and reaction time across positions. A group comparison demonstrated that there is a stronger effect of serial dependence in MCI patients compared to control groups in terms of reaction time (p-value = 0.028) but not

accuracy. The knowledge found from this study can be used to understand mechanisms of pathophysiologic change in MCI patients. Further studies into this effect could be a crucial step to develop a specific intervention for MCI patients.



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Poster

654. Working Memory

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Title: Repulsion-followed-by-attraction serial bias in 2-D continuous spatial perception

Authors: *M. LUO[1,2,3], H. ZHANG[1,2,3], H. LUO[1,2,3];
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Abstract: Serial bias refers to the automatic, systematic shifting of current perception by the preceding trial. Previous studies have shown an attractive serial bias in spatial perception, i.e., the perceived location tends to be attracted to that in the past trial. Meanwhile, these findings are based on the reproduction performance and the ongoing process before the final output remains unexplored. In the present study, we used mouse tracking to access the time-resolved spatial trajectory when human subjects performed a spatial location reproduction task. Specifically, subjects were briefly presented with a target shown at a random location in a 2D continuous space and needed to move the mouse cursor to the memorized target location, and their continuous mouse trajectory was recorded. First, we replicated the classical attractive serial bias, that is, the final clicked location of the mouse cursor was attracted to the target location in the previous trial. Most importantly, we observed a long-sustained repulsive serial bias in the mouse moving trajectory before the final reproduction output, advocating a 'repulsion-followed-by-attraction' serial bias temporal course in 2D continuous spatial perception. Furthermore, we showed that a physically presented distractor in the current trial could not induce the 'repulsion-followed-by-attraction' effect but displayed a late-repulsion profile only. Taken together, our results provide new perspectives supporting that serial bias in spatial perception is indeed a dynamic process encompassing two stages: an early repulsion and a late attraction.

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Poster

654. Working Memory

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Topic: H.05. Working Memory

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Title: Representations underlying a novel, naturalistic spatial working memory task

Authors: *E. CHONG[1], V. PLATTNER[2], L. CALCATERRA[1], A. AKRAMI[3];
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Abstract: Spatial working memory (SWM) is the short-term maintenance and update of spatial information. SWM is important for a broad range of cognitive tasks that involve reasoning about physical objects and spatially-organized information, even in abstract non-spatial contexts. What

are the neural computations underlying SWM? We trained rats to perform a novel, parametric SWM task (pSWM), where visual stimuli are projected onto the floor of a large behavioral arena, and animals learn to maintain target locations in their working memory over a delay period and report it by moving toward and pausing at the remembered location. Our projection arena exploits the previously-reported tendency of rodents to attend to stimuli close to the ground, and their rapid acquisition of visual tasks involving such stimuli. Our paradigm additionally overcomes several key limitations of existing SWM tasks: target location is continuous as opposed to discrete, and can be systematically controlled to account for various possible heuristic strategies, including body posture and motor preparations. It also spans a large space within animals' field of view, and mimics rodent naturalistic behavior. We used silicon probes to record activity in parietal and hippocampal regions, revealing the neural representations that correspond to spatial working memory in continuous coordinates under naturalistic conditions.

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Poster

654. Working Memory

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Program #/Poster #: 654.06

Topic: H.05. Working Memory

Title: Investigating working memory capacity in a novel free moving setup for marmosets

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Abstract: Working memory allows for the maintenance and manipulation of information relevant to goal-oriented behaviours. Working memory systems in the cortex are defined by capacity limits, wherein the number of memoranda that can be maintained in the working memory buffer is limited, modality-specific and varies across species. Recently, there has been great interest in marmosets as a primate model for working memory and other executive functions. Marmosets possess a lissencephalic cortical mantle making it feasible to precisely interrogate cortical layer-specific physiology during cognitive processing. Moreover, marmosets have a comparatively shorter reproductive cycle, and the attendant tractability of genetic manipulation and generation of transgenic marmosets makes them an attractive model for the study of prefrontal working memory circuits. Studies have demonstrated that marmosets are capable of classic working memory paradigms such as delay match to sample and position task. However, working memory capacity in marmosets has not been studied extensively. In macaques and humans, visual working memory capacity limits are independent of the two visual

hemifields. Neuronal persistent activity encoding stimulus location is reduced with increasing working memory load and working memory representations are degraded progressively. Less is known about the effect of working memory load on layer-specific intracolumnar processing and intercolumnar interactions. We propose to study this in marmosets using multi-shank linear probes during the performance of working memory tasks with increasing loads. To accomplish this, we have developed a modified in-cage, freely moving system to train marmosets in cognitive behavioural tasks which will allow for wireless neurophysiological recordings (Rogue Research). We have trained 8 marmosets on a sequential non-match-to-position touchscreen task, where subjects choose the stimulus that was not presented on the previous iteration of stimulus presentation. Each iteration within the trial increases the number of items to be maintained in working memory (1,2 or 3 stimuli). Five of eight marmosets were successfully trained to perform this sequential non-match to position task. We find that there is a high degree of inter-individual variability in the acquisition and performance of the task. However, with training, performance and working memory capacity are augmented. Understanding the capacity limits in the marmoset working memory system will facilitate future electrophysiological and molecular studies to understand how the fundamental constraints on working memory systems operate.

Disclosures: T. Lo: None. **S. Vijayraghavan:** None. **E. Hachinski:** None. **L. Muller:** None. **J.C. Martinez-Trujillo:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

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Program #/Poster #: 654.07

Topic: H.05. Working Memory

Support: NIH Grant R01EY028746

Title: Signal intrusion explains divergent effects of visual distraction on working memory

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Abstract: Perceptual distraction distorts visual working memories. Earlier research has focused on response biases induced by perceptual distractors, suggesting working memory representations are shifted towards distractors. However, recent research has shown divergent distraction effects on multiple forms of memory errors, including memory biases, reduction of memory precision, and increases in guess responses, depending on the similarity of the target and distractor. Distractors that are similar to the target often lead to response biases towards distractors, but they can also increase the precision of target responses and reduce the rate of guessing. As the target-distractor similarity decreases, distractors can lead to decreased memory precision and increased guessing. Based on these findings, perceptual distractors are sometimes beneficial and sometimes detrimental to working memory performance. Such divergent findings

could exist if sensory interference is driven by different mechanisms in different contexts. Here, we propose a novel distractor signal intrusion model, an extension of the target confusability competition (TCC) framework, to reconcile the discrepant results of perceptual distraction. We hypothesized that sensory interference, in all instances, is driven by intermingling the target memory signal and an intruded distractor signal. We tested this distractor signal intrusion model against classical memory signal shift models and models that combine both memory signal shifts and distractor signal intrusions. Through data simulations, we found that the distractor signal intrusion model can explain effects in memory bias, memory precision, and guesses using a single mechanism. Model comparisons showed that this model had a superior fit to working memory error distributions from four experiments (N = 120, age range 18-23 years, 77 females) compared to five other candidate models. Furthermore, with the distractor signal intrusion model, we revealed that the effects of perceptual distraction decreased along with the target-distractor similarity, as predicted by the sensory recruitment hypothesis. Together, these results suggest that perceptual distractors affect working memories through a unified mechanism of signal intrusion, rather than by multiple mechanisms that target different aspects of working memory performance.

Disclosures: Z. Zhang: None. **J.A. Lewis-Peacock:** None.

Poster

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Program #/Poster #: 654.08

Topic: H.05. Working Memory

Support: 5TU19ActNS107616
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Title: Prefrontal dopamine and serotonin in a novel self-ordered working memory task for mice

Authors: *B. GAMALLO LANA[1,2], P. LEONE[1], F. ARTIGAS[2], A. C. MAR[3];
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Abstract: Working memory (WM) is defined as the ability to temporarily maintain and manipulate information in order to guide behavior and higher-level cognition. WM deficits are central to many neuropsychiatric disorders, and are not improved by available pharmacotherapies. The integrity of the prefrontal cortex (PFC) and monoamine signaling are important modulators of successful WM performance, but the precise mechanisms are unknown. Quantitative assessment of cognitive performance is increasingly assessed by touch screen methods. The human CANTAB Spatial Working Memory test (SWM) is a widely used

touchscreen implementation of the classic spatial Self-ordered Pointing Test. Here, we developed and validated a rodent analog of the SWM - the rodent Self-Ordered Working Memory task (rSOWM) - for both rats and mice. On each trial of the rSOWM, visual stimuli are presented at locations along a horizontal grid on the screen (presentation phase). Animals then freely select any stimulus (choice phase) and are rewarded each time they select a unique visual stimuli location (reward phase). Re-selecting previously visited stimulus locations within a trial results in a timeout period that delays opportunity for the next reward. Multiple trial types were presented randomly to animals in each session, which varied in stimulus number (2-4), delay (0-6s) and spatial location/separation. We further examined the relationship between rSOWM task performance and changes in dopamine or serotonin dynamics within the medial PFC through expression of genetically encoded GRAB sensors and fiber photometry.
Performance was measured by the number of errors and perfect trials and was sensitive to parametric manipulations that impact WM performance in humans. Task performance in both mice and rats was decreased as the memory load and difficulty level was increased: larger stimulus number (e.g., 3 vs. 2), delay (6 vs. 0s) and spatial separation (large vs. short) between the visual stimuli. Measures of entropy and sequencing revealed that rats and mice developed preferential order responding strategies especially in the easier parameters of rSOWM. A forced-choice ordered probe also revealed that animals present higher performance when they are allowed to strategically order their choices. Dopamine and serotonin dynamics in PFC showed differential modulation during the distinct task phases of the rSOWM.
Our results suggest that rSOWM is a robust rodent analogue of human WM paradigms providing with a powerful translational tool for elucidating the mechanisms of WM and executive function and for improving preclinical drug discovery efforts.

Disclosures: **B. Gamallo Lana:** None. **P. Leone:** None. **F. Artigas:** None. **A.C. Mar:** None.

Poster

654. Working Memory

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Topic: H.05. Working Memory

Support: DST-Cognitive Science Research Initiative (DST/CSRI/2017/271)

Title: Olfactory matching reveals optimized time for accurate decisions

Authors: *R. BHOWMIK, M. PARDASANI, S. MAHAJAN, A. S. BHATTACHARJEE, S. KONAKAMCHI, S. PHADNIS, T. MUSTAFA, E. MCGOWAN, P. SRIKANTH, S. D. MARATHE, N. M. ABRAHAM;
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Abstract: Matching of various sensory stimuli involves neural signaling at the periphery as well as higher cognitive functions. Different decision processes such as detection and discrimination,

and holding the perceived information are involved during this course of action. In the context of increasing reports of olfactory dysfunctions under infectious and non-infectious disease conditions, establishing precise methods for quantifying olfactory fitness has become an emerging need. To probe sensory and cognitive functions involving olfactory system, we have developed a novel olfactory matching paradigm using an automated custom-built olfactory-action meter. With precise and consistent odor delivery and real-time data analysis, our system automates the entire process without any intervention of the experimenter, making it usable in clinical conditions. We have optimized all experimental parameters by quantifying olfactory detection and matching abilities in over 300 healthy subjects. With a mean detection accuracy around 90%, we observed significantly better olfactory matching performance for simple odors, in comparison to complex odor mixtures. Odor matching accuracy remained unaltered across varying inter-stimulus intervals. However, the olfactory matching time shown by the subjects for correct responses were significantly lower than the incorrect responses. This optimized paradigm offers quantification of sensory and cognitive deficits under various neurological disorders with olfactory impairments.

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Poster

654. Working Memory

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Topic: H.05. Working Memory

Support: NIH training grant FIC/NIH D43 TW001140

Title: Hippocampal neuronal density in a rat model in the neurocysticercosis disease

Authors: *L. BAQUEDANO SANTANA[1], E. G. BERNAL TERAN[2], M. R. VERASTEGUI[3]; [1]Univ. Nacional Mayor de San Marcos, Lima, Peru; [2]UNIVERSIDAD PERUANA CAYETANO HEREDIA, LIMA, Peru; [3]Infectious Dis. Lab. Research-LID, Univ. Peruana Cayetano Heredia, Chaclacayo, Peru

Abstract: Neurocysticercosis (NCC) is a disease produced by larvae of Taenia solium in the central nervous system. Parasites that have settled in the brain may lead to pleomorphic manifestations, like seizures, headaches, and cognitive impairment. Cognitive impairment may arise from an interaction of multiple factors between number, localization, vascular lesion, and local inflammation response of cystic lesions disrupting frontal-parietal-temporal networks related to intellectual functioning. However, the exact pathogenesis of cognitive dysfunction is controversial and furthermore, histological studies of the hippocampus have not been sufficiently elucidated in this disease. The aim of this study was evaluate the hippocampal neuronal density

in rats with neurocysticercosis presenting a cyst in the hippocampus, an area directly related to memory. This research was an experimental study conducted in male and female rats of Holtzman strain divided in three groups: rats with cyst in hippocampus, rats with cyst located in non-hippocampal area and control (n = 4, n = 4, n = 9, respectively). Parasitic infection was developed by intracranial inoculation of *T. solium* oncospheres in 14-days-old rats. Then, rats were sacrificed at twelve months post-inoculation, and histology was used to identify the presence of parasites (cysts) in the brain. H&E stain and Anti-NeuN immunostaining were used to evaluate the NeuN-positive cells of CA1. Hippocampal neuronal density in rats with cyst in hippocampus and rats with cyst located in non-hippocampal area was significantly different than the control group ($p<0.05$). The neuronal density in rats with cyst in hippocampus was 57 n°/field, rats with cyst located in non-hippocampal area was 72 n°/field, and control group was 83 n°/field. This study supports the relationship between neurocysticercosis disease and hippocampal neuronal density. Rats experimentally infected with NCC are a valuable animal model to evaluate the alterations of hippocampus and possible relationship with cognitive impairment.

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Poster

654. Working Memory

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Topic: H.05. Working Memory

Support: NRF-2018R1A4A1025891
NRF-2021R1F1A1052020

Title: Multiple stages of visual working memory distinctly contribute to the bias-variability pattern in delayed orientation estimation

Authors: *D.-G. YOO, H. GU, J. LEE, S. KIM, M. CHOE, H. LEE, H.-J. LEE, J. LIM, S.-H. LEE;
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Abstract: One primary goal of the brain is to estimate the true states of world events based on sensory information, even when such information is no longer available in the sensory apparatus. In such a situation, distributions of human estimation errors are far from homogeneous across stimuli. For example, in delayed orientation estimation tasks, estimation bias (center of an error distribution relative to the true value) peaks around orientations near the cardinal orientations (repulsion from the cardinal) whereas estimation variability (width of an error distribution) peaks around the oblique orientations. This bias-variability pattern across stimuli (BVAS) has become a benchmark phenomenon that must be explained by any healthy account of visual working

memory (VWM). The BVAS may arise as the brain utilizes natural stimulus statistics at both encoding and decoding stages of sensory information processing (NS-at-ED account). Alternatively, it may arise as the brain exploits category information when maintaining memoranda with a discrete-attractor mechanism during the delay period (DA-at-M account). These two accounts make distinct predictions about the location and pattern of the BVAS in the bias-variability space, which summarizes estimation performance with three statistics: bias, variability, and total errors. We compared these predictions against a set of human datasets from our and other labs. We found that both the accounts deviated from the observed BVAS. Specifically, the NS-at-ED account could capture the local geometry of the BVAS but understated the contribution of bias—relative to variability—to total errors. By contrast, the DA-at-M account could capture the relative contributions of bias and variability to total errors but outright failed to predict the local geometry of the BVAS, displaying variability peaking around the cardinal orientations. Given these incomplete yet compensatory successes and failures of the two accounts, we crafted an integrative account, where the brain not only utilizes the natural stimulus statistics at the encoding and decoding stages, as posited by the NS-at-ED account, but also drifts memoranda towards discrete attractors that are located to exploit categorical knowledge at the stage of memory maintenance, as posited by the DA-at-M account. We confirmed that our newly proposed account well captured the observed pattern of the BVAS, both in the local geometry and the relative contributions of bias and variability. Our findings suggest that errors in VWM have multiple origins encompassing the encoding, maintenance, and decoding stages and that the brain utilizes different types of knowledge over those stages.

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Poster

654. Working Memory

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Topic: H.05. Working Memory

Support: JHU80037721

Title: Multimodal brain imaging of methylphenidate treatment in patients with ADHD

Authors: *R. WISEMAN, P. BARKER, W. CLARKE, K. BIGOS;
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Abstract: Attention deficit hyperactivity disorder (ADHD) impacts a significant number of adult patients, with a small percentage receiving an accurate diagnosis and/or proper treatment. The goal of this double-blinded, placebo-controlled, crossover study was to identify ADHD-related signatures in the brains of adult patients and examine how brain activity, metabolites, and cognitive performance are altered with the commonly prescribed stimulant medication

methylphenidate. Participants were 20-36 years old, non-smoking, right-handed, not actively taking a stimulant medication, and had no other Axis 1 psychiatric disorders. We combined task-based 3T functional magnetic resonance imaging (fMRI) with high-resolution 7T magnetic resonance spectroscopy (MRS) to examine brain activity and brain chemistry, respectively. Two main fMRI tasks were utilized - the N-back working memory task and the flanker attention task to evaluate response inhibition. A standard cognitive battery including the NIH Toolbox Cognitive Battery was administered along with the Connors Adult ADHD Rating scale (CAARS), a self-report survey of ADHD symptoms. As expected, methylphenidate level correlated with frontal cortical activity during working memory ($p=9.9e10^{-5}$, slope=0.08073 $r^2=0.932$). The ADHD index, a CAARS measure of symptom severity, was shown to be sensitive to a single dose of methylphenidate (p=0.021), and drug level positively correlated with frontal cortical activity during working memory (p=0.0227, slope=6.165, $r^2=0.6067$). Increased glutamate levels in the anterior cingulate cortex and the dorsolateral prefrontal cortex (dlPFC) were associated with positive changes in composite cognitive function score (p=0.0047, slope=30.89, $r^2=0.95$) and fluid cognitive composite score (p=0.0066, slope=18.91, $r^2=0.94$), respectively. We also observed negative correlations between age and both processing speed (p=0.0171, slope=-2.266, $r^2=0.8852$, uncorrected scores) and dlPFC glutamate levels (p=0.0339, slope = -0.091, $r^2=0.82$). The novelty of this study is in the combination of approaches used to probe ADHD neural pharmacology. By combining functional measurements with high-resolution spectra of key brain metabolites and cognitive data, we created a richer picture of how stimulant medications impact the brain of those with ADHD. The fact that we were able to capture significant and synchronous changes in cortical activity and glutamate levels suggests that multimodal brain imaging may be a viable, noninvasive tool for ADHD drug development efforts.

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Poster

654. Working Memory

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Program #/Poster #: 654.13

Topic: H.05. Working Memory

Support: 180 Grader Nord WP3

Title: Higher physical fitness at age 18 improved working memory later in life in women, but not in men. A Norwegian Armed Forces Health Registry and HUNT study.

Authors: *P. STRANDHAGEN[1], L. REITLO[1], D. SOKOLOWSKI[1,2], A. K. HABERG[1]; [1]Dept. of Neuromedicine and Movement Sci., NTNU, Trondheim, Norway; [2]Dept. of Radiology and Nuclear Med., St. Olavs hospital, Trondheim, Norway

Abstract: Introduction: Physical activity and fitness have repeatedly been associated with better cognitive function, but most of the research has been conducted on patient populations, sedentary people and students. The aims of this study were to assess 1) the association between physical fitness and general cognitive ability at age 18, and 2) whether physical fitness in youth predicts cognitive abilities later in life. Additionally, we investigated the relationship between cognitive test performance later in life and general cognitive ability measured at age 18. Method: Data on physical fitness and general cognitive ability at age 18 were from the Norwegian Armed Forces Health Registry (NAFHR) and linked to cognitive performance data later in life (0.8 - 55 years) from the general population study HUNT4. Two measures of physical fitness were used: aerobic fitness as 3000m running and explosive strength measured as standing long jump. The NAFHR general cognitive ability measure is a normed score across several tests. We selected five test scores from HUNT4: digit span backwards (DSB), semantic clustering from verbal list learning, digit symbol coding, visual memory immediate recall and pattern separation reflecting similar cognitive domains constituting the NAFHR cognitive ability. We used GLM for the analyses. Interactions between sex and physical measure were included in all models. Results: A total of 1038 participants (10% women) were included. Participants were equally divided across all fitness levels. 1) Neither aerobic fitness nor explosive strength were associated with general cognitive ability measured at the same time. 2) No main effect was present between running and jumping and the five cognitive measures from later in life. However, significant interactions were uncovered between sex and running on DSB ($F = 7{,}375$, $p < 0.01$), and sex and jumping on DSB ($F = 22{,}715$, $p < 0.05$) with women who run faster or jump longer performing better at DSB. For men, physical fitness level did not influence performance. Finally, the cognitive measures predicted the general cognitive ability measure explaining 22.0% of the variance ($R^2_{\text{Adjusted}} = 0{,}220$, $p < 0.01$). Discussion: We found no relationship between fitness and general cognitive ability in the general Norwegian population at age 18. However, there was a future predictive value of fitness on DSB, but in women only. Since our population ranged across all fitness and cognitive ability levels, the results are not biased in a particular direction which might have been the case in previous studies. The sex difference for women on DSB reveals the importance of investigating both sexes to understand cognition and how to influence it.

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Poster

654. Working Memory

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Title: ‘Dynamic perturbation’ alters sequence working memory in human brains

Authors: *J. LI, H. LUO;
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Abstract: Temporarily storing a sequence of items in working memory (WM) is important in many cognitive processes and has been posited to rely on short-term neural plasticity (STP) principles whereby multiple items and their ordinal relationship are maintained in the WM network. Our previous work (Li et al., Progress in Neurobiology, 2021) has successfully developed a new 'dynamic perturbation' approach to manipulate the relative memory strengths of memorized items, via presenting flickering color probes with specific temporal associations in their respective luminance sequences during retention. Meanwhile, the neural underpinnings for 'dynamic perturbation' remains lacking. In the present work, we recorded Electroencephalography (EEG) activities on human subjects when they performed the same sequence working memory task with 'dynamic perturbation' applied during retention. Crucially, we presented a neutral PING stimulus after the perturbation to reactivate the neural representation of memorized items and calculated the neural index of the recency effect. Our results showed that the baseline condition elicits a prominent neural index of recency effect, while the synchronized condition disrupts the neural index of recency effect, consistent with behavioral findings. Taken together, we provide new neural evidence supporting that the 'dynamic perturbation' applied during the delay period indeed alters the relative memory strength of a sequence of items retained in WM.

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Poster

654. Working Memory

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Title: Precision of visuospatial working memory reports in adolescent monkeys

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Abstract: The precision of human visual working memory improves in childhood and adolescence [1] but the underlying neural mechanisms are not well understood. Here, we

addressed this question in non-human primates by studying response errors in a visuospatial delayed response task. Data were collected from 8 monkeys tested in the course of their adolescent development (ages 3.4-6.2 years). Monkeys reported the location of a memorized cue with a saccadic eye movement after memory delays of 1.5 or 3 seconds, and we studied the accuracy and precision of responses around the target location. Similar to humans, monkeys displayed increasing memory precision as they matured into adulthood. This was significant for 4 out of 8 monkeys individually and globally for the population (linear mixed model with continuous ages $p<0.05$). We then assessed systematic accuracy biases by computing inhomogeneities in response dispersion around targets across different target locations. Our data did not reveal any developmental trend in this measurement of accuracy biases (paired t-test for pre vs post maturation variation, $p>0.9$), suggesting that their development is decoupled from that of precision random errors. Finally, we also measured history biases in these monkeys, and more specifically serial biases. We found that monkeys showed a variety of attractive and repulsive serial biases, with four monkeys presenting predominantly repulsive and four monkeys displaying attractive serial biases. These biases too were fairly stable over this time (linear mixed model with continuous ages $p>0.4$), suggesting that serial biases, as accuracy biases, do not evolve developmentally following the progression of visual working memory precision improvement. In sum, our data shows that single-item working memory precision evolves gradually over the course of development into adulthood for monkeys, as it had been reported for humans, and does so independently of other systematic and history biases that affect visual memory reproduction. This suggests that different brain circuits are implicated in generating delay-dependent random errors and systematic biases in working memory, and/or that local-circuit mechanisms responsible for these memory distortions undergo different developmental trajectories. The parallel finding of increasing working memory precision in monkeys and humans opens the door to investigating the neural bases of this process through neurophysiology interrogations in monkeys.

[1] Heyes, S. Burnett, Nahid Zokaei, and Masud Husain. "Longitudinal development of visual working memory precision in childhood and early adolescence." Cognitive Development 39 (2016): 36-44.

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Poster

654. Working Memory

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Support: OHSU Physician Scientist Award
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Title: Extrapolation of behavioral strategy using motion tracking

Authors: *L. BARTLETT[1], A. SONNEBORN[2], A. I. ABBAS[3];
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Abstract: Modern behavioral neuroscience studies often rely on tasks that can be performed successfully using more than one strategy. As a result, it is often difficult to infer how an animal is performing a given task. Many studies do not examine movement data in way that can uncover the underlying strategies used throughout a particular task by a given animal, which may lead to biased or overgeneralized conclusions. We provide an example remedy to this problem using DeepLabCut - an open-source pose estimation toolbox - to extract movement features such as spin direction, relative body positioning, and velocity during key task points. We demonstrate here that through movement data alone, it is possible to identify several unique strategies used within a single cohort of mice, which all lead to similarly successful task performance. Surprisingly, we also found that the manner in which the task was completed was trial-type dependent, suggesting that individuals readily switch between different strategies within a session. Importantly, while results from machine learning analysis of neural activity and trial outcome (correct vs incorrect) could be interpreted as animals holding retrospective spatial information in mind to successfully perform the task, analysis of behavioral data indicated that animals were more likely using a prospective spin-based strategy to perform the task. We therefore conclude that future research should carefully consider behavioral task design in order to constrain the variety of potential strategies, complemented by machine learning techniques to better connect neural activity to specific aspects of behavior.

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Poster

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Title: Alternative splicing mechanisms relevant to stress and aging

Authors: *M.-L. WONG, E. CHIN, Q. MA, H. RUAN, C. CHIN, J. LICINIO;
Psychiatry, SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Genes critical to brain function and neuronal differentiation exhibit alternative splicing. Therefore, alternative splicing mechanisms are especially critical in the brain. CWC22 (spliceosome-associated protein CWC22) is critical for spliceosome assembly. The spliceosome is large RNA and protein complex that executes pre-mRNA splicing. We have investigated whether CWC22 contribute to neuronal dysfunction in stress, aging and in an Alzheimer's disease (AD) mouse model. We used C57BL/6 of different ages, and 5X mice. We submitted to chronic restraint stress (CRS) or intraventricular neonatal viral vector injection to knockdown or overexpress CWC22. Behavioral tests assessed behavior despair, anxiety, anhedonia, and cognitive functions. Brain tissues were used to investigate CWC22 levels using Western blots, immunohistochemistry, and RT-PCR. Primary hippocampal neurons were transduced with CWC22 knockdown or control lentivirus and studied with whole-cell patch clamp recordings. CWC22 is highly expressed in many brain regions, in the neuronal cell body layer and dendritic field areas. CRS induced behavioral changes, including anxiety-like behavior (decreased time spent in the center of the open field; $P<0.001$), anhedonia (decreased sucrose preference; $P<0.001$), and behavior despair (increased immobility in the forced swim test; $P<0.001$) in comparison to non-stressed mice (n=12-14/group). Hippocampal CWC22 levels were decreased in comparison to controls ($P<0.05$; n=8/group). CWC22 knockdown decreased mean mEPSC (miniature excitatory postsynaptic current) frequency and amplitude ($P<0.01$; n=20), and increased SYN1 + puncta and postsynaptic density protein 95-positive puncta numbers ($P<0.001$; n=4-15 neurites/condition) in vitro. Aged and 5xFAD mice had increased hippocampal CWC22 expression (both at $P<0.05$; n=5/group). Aged mice had decreased spontaneous alternations in the Y-maze ($P<0.05$; n>9/group) and exploration time in the novel object recognition test ($P<0.01$; n>9/group) than young mice. CWC22 over-expression significantly decreased the dendritic length ($P<0.001$) and branch number ($P<0.05$), and reduced mEPSC frequency and amplitude (both at $P<0.05$) in primary hippocampal neuron (n=12-30 cells/group). In summary, CWC22 regulation by stress, aging and AD may represent a newly-identified mechanism for underlying aberrant stress-induced splicing changes, contributing to stress-related behavioral, cognitive deficits, and pathophysiology. CWC22 is dysregulated in chronic stress, aging and AD. CWC22 has role in synapse formation and function, acting as a negative regulator of synaptic plasticity.

Disclosures: **M. Wong:** A. Employment/Salary (full or part-time):; eLife Sciences Publications, Ltd. **E. Chin:** None. **Q. Ma:** None. **H. Ruan:** None. **C. Chin:** None. **J. Licinio:** A. Employment/Salary (full or part-time):; Springer Nature.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.18

Topic: H.05. Working Memory

Support: Deputy Vice-Chancellor's Strategic Award for Research and Innovation, The University of Queensland

Title: Visual working memory is associated with white matter microstructure in the healthy human brain

Authors: *X. LI[1], D. RANGELOV[2], J. B. MATTINGLEY[2,3], L. K. L. OESTREICH[1], D. LÉVY-BENCHETON[1], M. J. O'SULLIVAN[1];
[1]UQ Ctr. for Clin. Res., The Univ. of Queensland, Herston, Australia; [2]Queensland Brain Inst., [3]Sch. of Psychology, The Univ. of Queensland, St Lucia, Australia

Abstract: Visual working memory, the ability to hold in mind and manipulate visual information over a short period of time, is critical to various daily activities. Research has shown that response accuracy in working memory tasks depends on the white matter microstructure of long-range association tracts, including the superior longitudinal fasciculus (SLF), inferior-frontal-occipital fasciculus (IFOF), and inferior longitudinal fasciculus (ILF). Here, we investigated whether microstructural properties in these tracts mediate the precision of memory representations, the retrieval of task-relevant representations, or other random processes during the task. We collected behavioral and diffusion-weighted imaging data from 72 healthy adult humans (36 females) aged 18-38 years. In the experiment, participants had to encode and maintain the representations of three gratings, varying in orientation and location, and to reproduce on a continuous response scale either the orientation or the location of only one of the gratings. To separately characterize the memory precision, retrieval accuracy, and random guesses, we modeled the distributions of response errors using mixture distribution modeling. We reconstructed the bilateral IFOF and ILF and also the dorsal (SLF I), middle (SLF II), and ventral (SLF III) parts of the SLF using probabilistic tractography and quantified white matter microstructures of each tract using fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity. We extracted four orthogonal factors from the tractography data using principal component analysis, which reflected either the bulk mean magnitude of diffusivity or directional diffusion along axonal fibers in different clusters of tracts. We found that higher memory precision was associated with lower bulk diffusivity in all tracts. We also found that higher precision and higher retrieval accuracy were predicted by higher directional diffusion in a cluster of frontal-occipital tracts (bilateral SLF II, SLF III, and IFOF) in individuals with decreased directional diffusion in the right SLF II and right SLF III. Importantly, there is no association between random guesses and any white matter factors. Our findings reveal that the microstructural properties of white matter tracts connecting posterior and frontal brain regions mediate, in a functionally specific manner, the efficiency of visual working memory maintenance and retrieval in neurotypical adult humans.

Disclosures: X. Li: None. **D. Rangelov:** None. **J.B. Mattingley:** None. **L.K.L. Oestreich:** None. **D. Lévy-Bencheton:** None. **M.J. O'Sullivan:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.19

Topic: H.05. Working Memory

Support: ERC StG MEMCIRCUIT 758032

Title: Behavioral signatures of inter-individual variability in prefrontal cognitive functioning in freely moving mice

Authors: *V. HOHENDORF[1,2], R. C. BRINKMANN[2], S. N. JACOB[1];
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Abstract: In order to deal with a large variety of diverse tasks, the brain's cognitive control centers such as the prefrontal cortex (PFC) help us to represent, memorize and interpret sensory stimuli and orchestrate appropriate (re)actions. Many studies investigating cognitive behaviors use simplified task designs that are often reduced heavily in an attempt to understand specific components of complex behaviors and to increase reproducibility. However, even in highly controlled experiments, variability between individuals is frequently found. Trying to remove or average across behavioral variability has been criticized and may lead to results that are not representative and poorly generalizable. Here, we aim to leverage this variability to distinguish between individual strategies and extract neuronal signatures that are essential for cognitive processes. Mice were trained on a spatial working memory task (trial-unique, non-matching-to-location (TUNL) task) in a touchscreen chamber that allowed free movement, enabling them to elicit distinct behavioral strategies to solve a cognitively challenging problem. Importantly, training proceeded in two steps. First, the animals were trained on a delayed response task in which they could use the location of a sample stimulus to fully predict the correct location of the subsequently presented test stimulus. In the second step, the animals had to memorize the sample location without being able to predict the test location and prepare an action. Tracking of animal position and locomotion was performed using DeepLabCut. Preliminary results show that even with a small sample size we were able to characterize animals based on idiosyncratic behavioral signatures including distinctive running and turning behaviors. Faster mice with a more direct path towards the stimuli performed better in delayed response trials, but their average performance in working memory trials dropped to chance level across the first three sessions. In contrast, slower mice performed better in trials where they could not predict the test stimulus location. Together, these findings show that the two tasks have different behavioral demands that are met with distinct strategies by the animals.The in-depth description and analysis of behavioral patterns during cognitive tasks will enable us to decompose complex neuronal signals and potentially separate correlates of inter-individually varying strategic approaches from conserved coding principles, specifically, how the sample stimulus representation changes when behavioral demands dictate a shift from delayed responding to memorizing without the possibility of action preparation.

Disclosures: V. Hohendorf: None. **R.C. Brinkmann:** None. **S.N. Jacob:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.20

Topic: H.05. Working Memory

Title: Laboratory-based spatial memory testing in the aged minipig and domestic pig

Authors: *L. M. ALLEN[1], M. MOUSSA[2], *A. DELGADO[4], M. AGUIAR[4], K. LEE[5], T. J. JAROME[6], T. A. ALLEN[3];
[1]Psychology Dept, [3]Psychology, [2]Florida Intl. Univ., Miami, FL; [4]FIU, Miami, FL; [5]Univ. of Missouri, Columbia, MO; [6]Virginia Tech., Blacksburg, VA

Abstract: Pig (*Sus scrofa*) biology is similar to humans in multiple ways such as fat distribution, hair coverage, and organ systems including skin, lung, cardiovascular, metabolic, and gastrointestinal. The pig genome is three times closer to the human genome than that of the mouse (Walters and Prather, 2013, *Advancing Swine Models for Human Health and Diseases*). As such, pigs have been used in translational biomedical research for more than 40 years. However, pigs have rarely been used in behavioral neuroscience, and could benefit translational neurological research on disorders such as age-related cognitive decline and Alzheimer Disease. As part of a larger strategy in developing the pig for translational behavioral neuroscience, we have been developing multiple laboratory-based cognitive/behavioral tasks for pigs. Here we present data from Minnesota minipigs and domestic pigs (mixed-breed) each of which present distinct advantages for neuroscience. For example, the minipig has a more manageable weight across their lifespan, while the domestic pig has a more accessible frontal cortex. Specifically, subjects were minipig (National Swine Resource and Research Center) and the domestic pig (Wallister Pork) that differed by age (0.5-6.5yrs) to assess viability and differences across cohorts in a classic spatial delayed alternation paradigm. The spatial alternation task is a staple in rodent behavioral neuroscience and responsible for much of what is known about the neurobiological mechanisms of learning and memory. Spatial memory was tested in a large, fully automated T-maze (17’ x 13’) with a start box, choice points, automatic rewards (flavored pellets), and return arms separated by guillotine doors. First, we tested the ability for pigs to acquire spatial memory by assessing trials to criteria (3 days at >75% accuracy). We found that younger domestic pigs (n=7, 0.5-1.5yrs) reached criteria quickly over 7 training sessions. By contrast, the older minipigs (n=2, 1.5-6.5yrs) demonstrated shallower learning curves; the 1.5yr minipig required 12 training sessions, while the 6.5yr counterpart needed 18 sessions. Next, we tested spatial working memory by introducing various randomly presented delays (5, 60, 120, and 240 sec) which provides a memory decay function. Here, we found that younger pigs performed well declining from 95-63% accurate across delays, while aged minipigs had a slight decrease in overall performance, but similar decay rate from 85-60% accurate across delays. We are continuing to test aged minipigs in order to see if spatial memory differences vary as a function of age, or reflect other cohort differences.

Disclosures: L.M. Allen: None. **M. Moussa:** None. **A. Delgado:** None. **M. Aguiar:** None. **K. Lee:** None. **T.J. Jarome:** None. **T.A. Allen:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.21

Topic: H.05. Working Memory

Title: Temporal regularity facilitates auditory working memory

Authors: *S. TIAN, Y.-A. CHENG, H. LUO;
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Abstract: Temporal regularity facilitates auditory working memory
Suizi Tian, Yu-ang Cheng, Huan Luo
School of Psychological and Cognitive SciencesIDG/McGovern Institute for Brain SciencePeking University
Temporal regularities are known to facilitate perception but whether and how it modulates working memory of auditory sequence remains unclear. In our behavioral experiment, human subjects were instructed to memorize a sequence of piano tones presented in either a rhythmic or arrhythmic way. After a maintaining period, the same tone sequence with one tone altered in pitch was presented, and subjects reported whether the shifting pitch was higher or lower than that of the memorized tone sequence. We employed a hierarchical drift-diffusion model to characterize the memory performances. Our results show that temporal regularity facilitates memory performances only when the number of tones in the sequence exceeds working memory capacity. Specifically, for short sequences with 4 tones, rhythmic and arrhythmic tone sequences showed similar performance. Importantly, for the longer sequences with 7 and 10 tones, rhythmic presentation improved the drifting rate of perceptual judgment compared to the arrhythmic condition. Taken together, temporal regularity improves auditory working memory capacity, presumably by distributing attention more efficiently on memorized items during encoding or enhancing memory consolidation through neural oscillations.

Disclosures: S. Tian: None. **Y. Cheng:** None. **H. Luo:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.22

Topic: H.05. Working Memory

Title: Dopamine d1 receptor-mediated regulation of Per1, Per2, Clock, and Bmal1 expression in the suprachiasmatic nucleus in adult male rats

Authors: *S. MESGAR, A. HAGHPARAST;
Neurosci. Res. Ctr., Shahid Beheshti Univ. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract: Abstract In mammals, the master circadian clock, located in the suprachiasmatic nucleus (SCN), receives direct input from the retina coordinates the timing of clocks in extra-SCN brain regions. SCN regulates the circadian rhythms via clock genes. SCN also receives non-photic inputs like dopaminergic (DA) afferents. It is known that any dysregulation of biological rhythms could lead to certain diseases in human beings. Dysfunction of SCN and changes in its afferents including photic and non-photic inputs might be the reasons. To study the effect of DA inputs on clock genes the present study designed. SKF38393 used intraperitoneally as dopamine D1 agonist. Twenty-eight adults male Wistar rat divided in four trial and control groups receiving SKF38393 and saline as vehicle for SKF respectively. By using real time PCR, the expression of Per1, Per2, CLOCK and BMAL1 genes was studied. Wheel running activity studied by running wheel apparatus. Following SKF treated the expression of clock genes changed significantly comparing to saline treated animals. SKF injection also increased the running wheel activity during night significantly. Based on our findings, any manipulation in dopaminergic afferents to SCN nuclei i.e., administration of exogenous dopamine in this study, is able to change the expression of clock genes, subsequently affect the biological rhythms and the animal behaviors. Accordingly, we believe that the increase of the growing evidence of dopamine's influence and role on circadian rhythms may lead to new treatments including pharmacological agents directed at relieving the various symptoms of circadian rhythm disruption. **Keywords:** Circadian rhythm; Dopamine D1 receptor; Suprachiasmatic nucleus; Per1; Per2; CLOCK; BMAL1; SKF38393

Disclosures: S. Mesgar: None. **A. Haghparast:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.23

Topic: H.05. Working Memory

Support: RF1 AG060754

Title: Intermittent stimulation of the nucleus basalis improves working memory in aged monkeys

Authors: *S. CHUNG[1], J. BAVA[2], Z. WANG[1], C. GARIN[2], K. CLEMENCICH[2], K. PENNINGTON[3], S. K. BICK[4], D. J. ENGLOT[2,4], D. T. BLAKE[3], C. CONSTANTINIDIS[1,2,5]; [1]Neurosci., [2]Biomed. Engin., Vanderbilt Univ., Nashville, TN; [3]Neurol., The Med. Col. of Georgia, Augusta, GA; [4]Neurolog. Surgery, [5]Ophthalmology and Visual Sci., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Degeneration of the basal forebrain cholinergic system is a key component in Alzheimer's disease (AD) and age-related cognitive decline. Accordingly, cholinesterase inhibitors are frontline medications and can, at least temporarily, improve symptoms in AD

patients. The Nucleus Basalis (NB) of Meynert is the source of neocortical cholinergic innervation in humans and non-human primates. Using Deep Brain Stimulation to target the NB presents an alternative means of increasing cholinergic activity. Here we tested whether NB stimulation improves working memory in aged monkeys. Rhesus monkeys (*Macaca mulatta*) 24-33 years of age were trained on a delayed match-to-sample task and reached asymptotic performance. Adaptive control of delay durations allowed tracking of threshold delay periods for which the animals could achieve 79% correct choices in each session. Monkeys were implanted with electrodes targeting the NB and an Implantable Pulse Generator (IPG) under the skin. A micro-welded pigtail connected the IPG device and stimulation electrodes. Daily, the subjects received unilateral intermittent 60-Hz stimulation, lasting 20 seconds every minute, in a 1-hour window. The choice of intermittent stimulation was critical as our prior monkey results have shown that continuous stimulation is ineffective. Two female animals (age 24 and 28 years) completed an 8-week period of unilateral stimulation. Significant increases in the average threshold delay durations were observed after stimulation, of 2.58 seconds (permutation test, $p = 1.5E-3$) and 1.87 seconds (permutation test, $p = 8.2E-3$), for the subjects, respectively. A generalized linear mixed-effect model showed that this change in performance was significantly larger (interaction effect, $p = 1.6E-3$) than what was expected from unstimulated counterparts ($n = 5$) across the same time frame. PET scans with FluoroDeoxyGlucose tracers were carried out after 8 weeks of NB stimulation. The Standardized Uptake Value ratio (normalized on the average of the cerebellar cortex) was significantly increased in the stimulated site, compared to the unstimulated one. Our results show that intermittent stimulation of NB improves working memory functions and brain metabolism in a non-human primate model of age-related memory decline.

Disclosures: **S. Chung:** None. **J. Bava:** None. **Z. Wang:** None. **C. Garin:** None. **K. Clemencich:** None. **K. Pennington:** None. **S.K. Bick:** None. **D.J. Englot:** None. **D.T. Blake:** None. **C. Constantinidis:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Boston Scientific provided IPGs and supporting materials.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.24

Topic: H.05. Working Memory

Support: UNAM DGAPA PAPIIT IG300121

Title: Metamemory mediates the effects of age on episodic and working memory across the adult lifespan

Authors: ***S. CANSINO**, F. TORRES-TREJO, C. ESTRADA-MANILLA, S. RUIZ-VELASCO;

Lab. NeuroCognition, Nat Autonomous Univ. of Mexico, Univ. Nac Autónoma de México, Mexico City, Mexico

Abstract: The aim of the study was to establish whether the effects of age on episodic and working memory are mediated by the knowledge, emotions or beliefs we have regarding our own memory. We examined episodic memory and working memory through computerized tasks performed by a lifespan sample of 1554 healthy adults. Seven metamemory traits were measured with the Metamemory in Adulthood (MIA) questionnaire. Separate structural equation modeling analyses were conducted to investigate potential metamemory mediators that intervened between age and the accuracy and speed of accessing information from episodic and working memory. The use of internal or external strategies mediated the effects of age on episodic memory. The perception of our own memory capacity and of the influence of anxiety on memory performance mediated the effects of age on working memory. Metamemory traits have the power to strengthen or weaken the course of episodic and working memory decline throughout adulthood.

Disclosures: S. Cansino: None. **F. Torres-Trejo:** None. **C. Estrada-Manilla:** None. **S. Ruiz-Velasco:** None.

Poster

654. Working Memory

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.25

Topic: H.05. Working Memory

Title: Ensemble representations in visual working memory

Authors: *K. RAAFAT, L. YE, K. K. SREENIVASAN;
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Abstract: Ensemble representations in visual working memory
Humans encode the ensemble statistics of their environment with astonishing speed and accuracy. Previous research has shown that, in addition to influencing perception, these ensemble representations can also influence visual working memory (WM) for individual items. This suggests that the encoding of ensemble representations may be a potential adaptive mechanism used to cope with the limited capacity of WM. However, little direct evidence exists for this functional connection between ensemble representations and WM. Here, we explored this relationship by examining whether the strength of ensemble bias was modulated by WM load. Specifically, we examined whether external load (set size) and internal load (WM capacity) affected the degree to which participants' memory reports were biased by the ensemble properties of their memories. We used a change-detection task to measure participants' WM capacity (k) and divided them into high and low capacity groups. Both groups then performed a task where they were presented with a memory display consisting of 4, 6, or 8 circles of different sizes. After a brief delay, they were cued to recall the size of one of the circles in the memory

display. We tested participants' memory using a 3-alternative forced choice report. The probe display contained three circles: one that was the size of the cued circle (the correct item), one that was the size of the ensemble mean of the circles in the memory display (the ensemble item), and one that was neither in the memory display nor the ensemble mean (the non-match item). Critically, neither the ensemble item nor the non-match item were in the original memory display, and both were equidistant in size from the correct item. When participants chose incorrectly, they were overwhelmingly more likely to choose the ensemble item over the non-match item, confirming that participants' memory reports were biased by the ensemble properties of the display. However, this bias was remarkably consistent as a function of set size and WM capacity. One possible explanation for this result is that participants' memories incorporated ensemble statistics at a low threshold of only a few items, resulting in a consistent ensemble bias regardless of internal or external WM load. These results may also indicate that rather than being a mechanism that only plays a role when WM is taxed, ensemble representations may instead be automatically computed and encoded alongside WM for individual items.

Disclosures: **K. Raafat:** None. **L. Ye:** None. **K.K. Sreenivasan:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

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Program #/Poster #: 654.26

Topic: H.05. Working Memory

Support: NIMH Grant R01MH122613

Title: Impaired working memory precision and distractor resistance following lesions to the human thalamus

Authors: *X. CHEN, K. HWANG;
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Abstract: The human thalamus likely has an important yet underspecified contribution to working memory processes. Anatomical and electrophysiology studies from animal models suggest that the thalamus may mediate the effects of top-down biasing signals on cortical processes. In the context of working memory, top-down biasing signals sustain working memory content and protect against distractor influence. Whether or not the human thalamus is involved in these top-down processes for working memory has been difficult to study. To explore whether the human thalamus is involved in top-down control of working memory, we examined the behavioral performance of thalamic patients, comparison patients, and healthy control subjects in a retro-cue working memory task. Thalamic patients had focal lesions in the medial and anterior thalamus, whereas comparison patients had lesions similar in size but spared the thalamus. In the task a pair of Gabor patches with different orientations were presented, with one of two patches

designated as the target required to be reported at the end of trial. Target presentation was followed by another pair of Gabor patches serving as distractors. We manipulated the similarity between distractors and targets, hypothesizing that the similarity will influence the degree of distractor interference. After the display of distractor, a neutral or valid cue was presented to examine the effect of top-down biasing on working memory precision. We modeled the behavioral data with a mixture-model (Zhang & Luck, 2008). In comparison patients, the valid cue improved working memory precision when the distractor was similar to the target. However, we found that this benefit of improving working memory precision from the valid retro cue was weaker in thalamic patients. Furthermore, thalamic patients showed less precise working memory performance overall. This result indicates that the human thalamus mediates the effect of top-down biasing signal for improving working memory precision and limiting distractor interference.

Disclosures: X. Chen: None. **K. Hwang:** None.

Poster

654. Working Memory

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Program #/Poster #: 654.27

Topic: H.05. Working Memory

Support: CIHR-IDRC-ISF #2558/18
CRCNS BSF#2015577
ERC #679253

Title: Is the dorsal hippocampus necessary for non-spatial short term memory?

Authors: *S. SOMECK[1], N. KATZ[2], E. STARK[1];
[1]Dept. of Physiol. and Pharmacol., [2]Tel Aviv Univ., Tel Aviv, Israel

Abstract: Short term memory (STM) involves neuronal activity in multiple brain regions. In rodents, the hippocampus is necessary for spatial STM, but it is unknown whether non-spatial STM requires intact hippocampal activity.
Here, we developed a novel paradigm which includes discrimination and STM tasks. The apparatus is a figure-8 maze with two pairs of motorized texture wheels, separated by a 120 cm track. Every wheel holds multiple textures, and the mice learn to associate distinct textures with left and right turns. In the STM task, textures are presented at the start wheels, requiring memory maintenance throughout the track. Four mice learned the discrimination task within seven testing sessions (median success rate, 83%; trials, 133). Six mice learned the STM task within nine sessions (67%, 96).
We previously found that the fraction of CA1 place fields near the choice and reward regions increases after learning the discrimination task, implying CA1 involvement (Someck el al., 2021; SFN 50:P821.10). To directly test whether CA1 activity is necessary for STM maintenance, we

injected viral vectors into the dorsal hippocampus bilaterally, expressing Jaws in pyramidal cells. Every mouse was implanted bilaterally with optical fibers coupled to red laser diodes. Presently, red light is being used to silence the pyramidal cells specifically during memory maintenance.

Disclosures: **S. Someck:** None. **N. Katz:** None. **E. Stark:** None.

Poster

654. Working Memory

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Program #/Poster #: 654.28

Topic: H.05. Working Memory

Support: Alfred P. Sloan Research Fellowship (TCS)
UCSB URCA Grant

Title: Awareness of the relative quality of spatial working memory representations

Authors: ***Y. LI**[1], T. SPRAGUE[2];
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Abstract: Working memory (WM) is the ability to maintain and manipulate information that is no longer accessible in the environment. The brain maintains WM representations over delay periods in population-level activation patterns across a broad swath of cortex (Curtis & Sprague, 2021). Because neural processing is noisy, the quality with which these neural codes encode remembered stimuli varies across trials. Behavioral and neural studies have shown that participants can accurately read out the quality of a single WM representation (e.g., Rademaker et al, 2012), and behavioral reports are related to aspects of the neural representation quantified with decoding techniques (Li et al, 2021; Geurts et al, 2022). Moreover, studies requiring participants to remember stimulus features (e.g., color) have shown that participants can introspect the relative precision of multiple WM representations (Fougnie et al, 2012; Suchow et al, 2017). However, whether this ability extends to WM for spatial locations remains unknown. Here, we employed a memory-guided saccade task to test the precision with which participants report a remembered spatial location when they were allowed to choose the most precise representation to report. Participants (n = 20) remembered either one or two spatial locations over a 3.5 s delay interval and reported the location of one item at the end of each trial with a saccade. On trials with two spatial locations, participants were instructed to either report the spatial location of a cued item (randomly chosen) or report the location of the stimulus they believed they remembered the best. If participants can accurately introspect the relative quality of the spatial WM representations, then recall precision will be better when participants can choose which representation to report. We found a significant improvement in precision ($t = 3.982$, $p = .002$) and increase in RT ($t = 3.685$, $p < .005$) when participants report their best-remembered item compared to trials in which they were cued which item to report. Further

analyses provided evidence that participants were monitoring the trial-by-trial fluctuations in the relative quality of multiple spatial WM representations instead of employing heuristics such as "report the first location that came to mind" or "report the left stimulus". These results support the conclusion that participants can accurately introspect the quality of neural WM representations for spatial position, consistent with previous observations for other stimulus features.

Disclosures: Y. Li: None. **T. Sprague:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

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Program #/Poster #: 654.29

Topic: H.05. Working Memory

Support: FODECIJAL 8237-2019

Title: Work memory deficit and hyperglycemia in diabetic rats is partially reverted by an anthocyanin extract from Hibiscus sabdariffa

Authors: *C. RUELAS MONTES, M. E. UREÑA-GUERRERO, N. A. POSADAS-RAMIRO, J. L. CASTANEDA-CABRAL, S. J. LOPEZ-PEREZ;
Biologia Celular y Mol., Ctr. Universitario de Ciencias Biológicas y Agropecuarias, Univ. de Guadalajara, Zapopan, Jalisco, Mexico

Abstract: Diabetes is a metabolic disorder and a global public health problem that has also been associated with memory impairment, as a probable result of neuroinflammation and oxidative stress induced by hyperglycemia (HYP), which could be controlled by naturally occurring compounds with high antioxidant capacity, such as anthocyanins. A good source of this type of molecules are plants, some of them commonly used for human consumption, such as *Hibiscus sabdariffa*. To explore the ability of a Hibiscus anthocyanin extract (EXT) to alleviate HYP and preserve short-term memory, male Wistar rats within body weight 228±14.9 g and blood glucose levels (BGL) 103±13.23 mg/dL were induced to HYP by a single i.p. injection of streptozotocin (STZ) at a dose of 55 mg/kg under fasting conditions. Only rats with BGL greater than 200 mg/dL 72 h after injection continued in the experiment. A subgroup of control (CTL) and HYP groups was selected to consume the EXT in drinking water (50 mg/kg/day) for 30 days during which body weight and BGL were measured every 10 days. Starting on day 31, average scape latency (ASL) was recorded for each group in a Barnes Maze (BM), and a discrimination index was assessed by an object recognition test (NOR). Data was plotted in mean and standard deviation, and differences among groups were analyzed using one-way ANOVA and Tukey *post-hoc* tests (α=0.05). On day 10 of the EXT consumption, 65% of the animals in the HYP group had BGL <200 mg/dL (109±37.8 mg/dL), this effect was temporal and on day 30 all animals had HYP again. In BM, rats with BGL between 450-550 mg/dL 72 h after STZ injection

(40%) had larger ASL than the CTL group in all sessions (global ASL 31.82±21.07s in CTL vs. 248±87s in high-HYP), while global ASL in rats with BGL between 250-425 mg/dL 72 h after STZ (60%) was closer than observed in CTL group (84.79±81.70s in low-HYP). For HYP+EXT group, global ASL was similar compared to CTL group (37.93±28.86s). In NOR, the discrimination index of HYP group (-0.034 ±0.48) was lower than in CTL group (0.618±0.15), and the HYP+EXT group had similar values to CTL. These results indicate that HYP has a significant impact on short-term memory acquisition and retrieval, which seems to depend on the individual BGL, with worse performance when levels were higher. Moreover, the anthocyanin extract consumption exerts a positive effect on the BGL control and short-term memory capacity, highlighting the possibility of its therapeutic use in *diabetes mellitus*.

Disclosures: C. Ruelas Montes: None. **M.E. Ureña-Guerrero:** None. **N.A. Posadas-Ramiro:** None. **J.L. Castaneda-Cabral:** None. **S.J. Lopez-Perez:** None.

Poster

654. Working Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 654.30

Topic: H.05. Working Memory

Support: Ratchadapiseksompotch Fund, RA64/021

Title: Long term memory outcome in transient global amnesia patients with restricted diffusion lesion in magnetic resonance imaging

Authors: *S. CHUNAMCHAI[1,2], N. MAHITTHAFONGKUL[3], C. CHUNHARAS[1,2];
[1]Cognitive Clin. & Computat. Neurosci. Lab, Dept. of Intrnl. Medicine, Fac. of Medicine, Chulalongkorn Univ., Bangkok, Thailand; [2]Chula Neurosci. Center, King Chulalongkorn Mem. Hospital, Thai Red Cross Society, Bangkok, Thailand; [3]Div. of Neurology, Dept. of Intrnl. Medicine, Fac. of Medicine, Chulalongkorn Univ., Bangkok, Thailand

Abstract: Transient global amnesia (TGA) resulting from reversible injury of the hippocampus has short term effects on both verbal and visual memory. However, evidence on long-term cognitive outcomes is still unclear due to various factors of hippocampal injuries, including size, site and number of lesions, which might play a role in memory outcome. We collected data from 26 TGA patients whose MRI brain showed restricted diffusion in the hippocampus compared with 26 age and sex matched controls. The Thai version of the Montreal Cognitive Assessment (MoCA) and other comprehensive neuropsychological battery tests were used to evaluate cognitive function in visual and verbal memory domains in immediate and delayed memory types. Baseline characteristics including age (62.8 ± 5.7 vs 61.4 ± 4.8, p = 0.338), education level (p=0.11) and MoCA score (26.12 ± 2.86 vs 26.92 ± 2.19, p = 0.258) were not different between group. In neuropsychological battery tests, TGA performs worse than controls in immediate visual memory tests (8.88 ± 2.16 vs 10.12 ± 1.7, t = 2.281, p = 0.027). Visual memory

reproduction recognition score was lower in TGA (4.85 ± 1.16 vs 5.46 ± 0.91, p = 0.037). Three-ways mixed ANOVA considering effect from group, memory domain and memory type shows better performance in control group, verbal memory test and immediate memory type (F = 4.19, p = 0.046, F = 4.284, p = 0.044, F = 31.236, p < 0.001 respectively). There was no interaction between the group of patients and memory type or memory domain. In subgroup analysis of TGA patients, right-sided and both sided lesion patients tend to perform better in delayed visual memory while left-sided lesion patients tend to perform better in verbal memory. This finding contrasts with the evidence of performance in neurocognitive function in patients with structural brain disease such as sequelae of stroke or epilepsy and could result from an unequal baseline of the hippocampal function. Higher function of the right hippocampus may cause better visual cognitive performance, consumes more energy, and thus leads to higher risk of injury. To investigate this hypothesis, further volumetric measure of the hippocampus and correlation with cognitive performance will be explored. Longitudinal neurocognitive function follow-ups in this group of patients will also be important in further observation of cognitive decline. In conclusion, transient global amnesia patients who have abnormal MRI brain signal have sequelae on long term memory outcome compared to controls. Progression of cognitive function declination and association with the characteristic of the hippocampal lesions should be evaluated.

Disclosures: **S. Chunamchai:** 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.; Ratchadapiseksompotch Fund RA64/021. **N. Mahitthafongkul:** None. **C. Chunharas:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.01

Topic: H.06. Social Cognition

Support: SIDB Grant R83880
Wellcome Trust Grant 207481/Z/17/Z

Title: Selective rescue of social alterations by early Lovastatin treatment in a rat model of Fragile X syndrome

Authors: *N. GARCIA[1], S. R. LOUROS[1], F. GOBBO[1], E. K. OSTERWEIL[1], R. G. MORRIS[2]; [1]Ctr. for Discovery Brain Sci., Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Ctr. for Cognitive and Neural Systems, The Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: *Aim*: Fragile X syndrome (FXS) arises from the silencing of the Fmr1 gene that abolishes the expression of the Fragile X mental retardation protein (FMRP). FMRP is a brain-enriched RNA binding protein that plays an essential role in translation and RNA transport. The

most notable clinical alteration of FXS is developmental delay, learning disabilities and social changes. This study examines the impact of Lovastatin, a drug used in the treatment of the hypercholesterolemia that inhibits Ras protein translocation, as a possible therapeutic approach for social alterations observed in a rat model of FXS.
Experiment: The study examined both social interaction and social dominance tests. There were 12 wild-type (WT) and 11 *Fmr1* KO (KO) Long-Evans male rats and 11 WT and 10 KO L-E male rats received Control/Lovastatin supplemented food respectively for 5 weeks from weaning until day 56. After treatment, we tested their sociability, social novelty and social dominance in a version of the 3 compartment Social Interaction Test (SIT, Crawley, *Ment. Retard. Dev. Disabil. Res. Rev*, 2004) and in the Tube Test (TT). The rats were used as "Enclosure rats (ENC)" in the SIT, with an additional 18 WT and 16 KO L-E male rats as "Free to Explore rats (F2E)" (n=78 total). Broadly, both sociability and social novelty were normal in KO rats, but we also observed that, in the sociability phase, KO F2E rats expended significantly less time than the WT F2E rats exploring the ENC rat when it was a KO rat under control food. This difference was not observed when the ENC KO rat received the Lovastatin treatment. In the TT conducted 7 months later, we confirmed that WT littermates were dominant over cage-mate KO rats that were under control food (Saxena et al, *Proc.Roy.Soc.B*, 2018), but the submissive status of the KO rats was rescued by Lovastatin treatment. When the TT was conducted again, but now between animals from different cages, control KO rats were again submissive, but Lovastatin restored this status also. Importantly, Lovastatin was no longer present at testing having only been given from day 21-56.
Implications and future perspectives: These findings point to a selective impact of early Lovastatin with minimal changes in Sociability but a striking and replicable rescue of an *Fmr1* KO-associated deficit in social dominance. Our observation of rescue in KO animals approximately 180 days after the end of a 5 week treatment constitutes an addition to the results from a 'what-where-which' recognition memory test by Asiminas et al, *Sci. Trns .Med.*(2019). Our findings could have translational relevance, as they are the first FXS treatment associated correction of social abnormalities in an animal model.

Disclosures: N. Garcia: None. **S.R. Louros:** None. **F. Gobbo:** None. **E.K. Osterweil:** None. **R.G. Morris:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.02

Topic: H.06. Social Cognition

Support: SIDB, Edinburgh - R83880
Welcome Trust Investigator Grant - 207481/Z/17/Z

Title: Ca^{2+}imaging of self and other in medial prefrontal cortex during social dominance interactions in a Tube-Test

Authors: *R. G. M. MORRIS[1], N. GARCIA[2], K. SAXENA[5], S. CHATTARJI[6], P. C. KIND[3], R. MITCHELL-HEGGS[4], S. R. SCHULTZ[7];
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Abstract: *Aim:* The study of social dominance interactions between animals offers a window onto the decision-making involved and an opportunity to examine changes in social behavior observed in certain neurogenetic disorders. This study used endoscopic calcium imaging in the prelimbic zone (PrL) of the medial prefrontal cortex (mPFC) to explore competitive social interactions. Following Kingsbury et-al, (Cell,2019), we focused on both neural correlates of an animal's own behavior and the behavior of the opposite/competitor animal.
Experiment: There were n=12 wild-type and *Fmr1* Knock-out Long-Evans rats (2 per cage). We targeted the GRIN lens for observing calcium transients at PrL during the tube-test following observations of its importance in social dominance (Wang et-al, *Science*,2011). Viral GCamp6f was first infused into PrL and then standard procedures followed for optimal imaging using the Inscopix system (Mukamel et-al, Neuron,2009). We observed 913 cells (mean = 77 per animal) active during distinct facets of tube-test behavior such as MOVE FORWARD, PUSH, RESIST, RETREAT, WITHDRAWAL and STILLNESS, and measured the stability within days and consistency across days of regions of interest (ROIs). Using a rigorous mutual information criterion, we observed that neural responses recorded in PrL showed unique correlations to these specific dominance-related behaviors. Inter-animal analyses revealed cell/behavior correlations that were primarily with an animal's own behavior, with the competitor animal's behavior, or with the coincident behavior of both animals (such as pushing by one and resisting by the other). The comparison of unique and coincident cells disentangles cell firing that reflects an animal's own, the competitor's specific behavior, and situations reflecting conjoint action. We observed that correlations are dynamic and can sometimes change across days, reflecting the plasticity of PFC.
Implications: A dominant animal cannot just unilaterally "decide" to be dominant - it may need to push to find out how the other animal will react. Such interactions fuel interbrain correlations between two animals. Beyond this, a social situation is one in which correlations between the neural activity in one brain and the decision-making and behaviour of the other animal is to be expected. Following this idea, studies of sociability and social recognition memory point to cognitive aspects of social interaction.

Disclosures: R.G.M. Morris: None. **N. Garcia:** None. **K. Saxena:** None. **S. Chattarji:** None. **P.C. Kind:** None. **R. Mitchell-Heggs:** None. **S.R. Schultz:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.03

Topic: H.06. Social Cognition

Support: JSPS KAKENHI 18H02712

Title: Visual reinforcement by conspecifics varies individually according to their social interests in common marmosets

Authors: *Y. YAMAZAKI[1], R. BRETAS[1], A. IRIKI[2];
[1]RIKEN BDR, [2]RIKEN BDR, Hyogo, Japan

Abstract: In social animals, appearance of conspecific individuals can be rewarding because they might convey various sources of information such as food availability, hazardous situations, and reproductive states. We examined the reinforcing effects of visual appearance of other conspecifics in the captive marmosets, using the apparatus with touch sensitive screens. Two pairs of subjects were trained to choose either of the figures which were simultaneously presented on the monitor. A response to one stimulus led to reinforcement by sweetened liquid only, whereas a response to the other one led to reinforcement by the liquid and the appearance of the conspecific, paired animal (partner) in the chamber located adjacent to the one with the experimental animal. Appearance of the animal was controlled by changing the transparency of the glass wall between the chambers. Baseline training sessions were conducted daily for each animal of the pairs: the same individuals appeared when they chose the option with appearance of the partner. Four training sets were conducted, and the reinforcement contingencies of the figures were switched in every set. They gradually differentiated the choice behaviour according to the outcomes with or without visual reinforcement. After completion of the training sequences, the subjects were tested in the sessions which non-partner individuals were presented when they chose the visual reinforcement option. The test results showed that the choices for the visual reinforcement option temporally increased when they saw unusual partners adjacent to their experimental chamber. The changes in choice pattern in the test sessions varied individually and could be interpreted by multiple reasons: visual reinforcement by presence of conspecifics, by possible reproductive partner, or by possible competitor for reproduction. Thus, the present experiments confirmed visual reinforcement by conspecifics in common marmosets, which needs to be examined by additional populations because of choice variability caused by diverse individual interests on others.

Disclosures: Y. Yamazaki: None. **R. Bretas:** None. **A. Iriki:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.04

Topic: H.06. Social Cognition

Support: NIH, NIMH R01MH117785
MH057414

Title: Laminar pattern of connections within the temporal lobe of the rhesus macaque.

Authors: *J. BAUTISTA[1], M. GARCIA-CABEZAS[4], B. ZIKOPOULOS[2], M. MEDALLA[5], H. BARBAS[3];
[1]Boston Univ., [2]Boston Univ., Boston, MA; [3]Hlth. Sciences, and Anat. and Neurobio., Boston Univ., Brookline, MA; [4]Dept. de Anatomía, Histología y Neurociencia, Univ. Autonoma de Madrid, Madrid, Spain; [5]Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: The entorhinal cortex (A28) is the gateway to the hippocampus, and it receives largely feedforward pathways from multimodal association areas. A28 is linked through reciprocal pathways with nearby perirhinal and temporal visual and auditory association cortices in the temporal lobe. The laminar organization of these pathways, which is relevant to the information flow for memory processing, is not well understood in primates. We studied connections within the temporal lobe by injecting bidirectional neural tract tracers in different areas: TS1, TS2, TE1, area 36, TPro and area 28 to determine their density and laminar distribution. These temporal areas can be categorized into three different cortical types based on their laminar architecture: the sensory association areas TS1, TS2 and TE1 have six layers (eulaminate), the perirhinal limbic areas TPro and area 36 have an incipient layer IV (dysgranular), and area 28 lacks layer IV (agranular). Our group has demonstrated a robust quantitative relationship between laminar architecture and patterns of connections, defined as the Structural Model for corticocortical pathways. Our results were consistent with predictions of the Structural Model, showing that (1) temporal areas that are similar in laminar architecture (within the same cortical type) are strongly interconnected; and (2) the laminar pattern of connections is dependent on differences in cortical laminar structure between linked areas. Thus, agranular A28 is more strongly connected with other agranular/dysgranular areas than with eulaminate cortices. Further, A28 predominantly sent feedback-like projections that originated in the deep layers and terminated in the upper layers of the target area, and received feedforward-like projections from areas of greater laminar differentiation, which emanated from the upper layers and terminated in the middle layers of A28. Compared to eulaminate cortices with more complex laminar architecture, dysgranular and agranular (limbic) areas have a greater expression of markers associated with circuit plasticity (e.g., GAP-43) and a lower density of markers of stability (e.g., myelin), which may render them more vulnerable to neurodegenerative and psychiatric diseases. Accordingly, understanding the laminar pattern of connections of agranular areas with other cortices within the temporal lobe may shed light on the signal propagation in this network and disruptions in neurologic and psychiatric diseases.

Disclosures: J. Bautista: None. **M. Garcia-Cabezas:** None. **B. Zikopoulos:** None. **M. Medalla:** None. **H. Barbas:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.05

Topic: H.06. Social Cognition

Support: NIH, NIMH MH057414
NIH, NIMH R01MH117785

Title: Comparison of the amygdala and hippocampal pathways in the nucleus accumbens shell in Rhesus macaques

Authors: *L. MARSHALL[1], H. BARBAS[2];
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Abstract: The nucleus accumbens (NAc) is critical for our ability determine the value of stimuli and strategically respond to current conditions, and receives projections from both the amygdala and hippocampus. While the amygdala projects across the NAc, the hippocampus primarily innervates the shell subregion of the NAc. The overlap in connections at the NAc shell position it to have a significant role in action selection with access to both emotional information from the amygdala and contextual information from the hippocampus. Behavioral studies in rodents have shown that these connections are necessary to distinguish the value of distinct cues based on spatial location and to enhance memory consolidation during emotionally significant events. Here, we mapped pathways from the basomedial/basolateral nuclei of the amygdala and the hippocampus of rhesus monkeys to NAc shell using anterograde tracers. Consistent with previous studies, labeled axons showed a diffuse projection from the amygdala and a more targeted projection to the medial aspect of the NAc from the hippocampus. Using an unbiased random sampling stereological technique, we estimated the density of labeled boutons from the amygdala and hippocampus at the medial and lateral aspects of the NAc shell. We found a higher density of hippocampal bouton in the medial NAc shell, but no significant difference in the density of amygdalar boutons between the medial and lateral shell. We also measured the major diameter of boutons from these two pathways. We found that the amygdala tended to have larger boutons than the hippocampus, which suggests stronger amygdalar synapses onto NAc neurons, because larger boutons are associated with higher probability of multivesicular release and a higher chance of activating the post-synaptic target. A comparison of the patterns of bouton density in the NAc shell, plus variations in bouton sizes from the amygdala and hippocampus, provide insight into the relative influence that these two projections have on NAc shell activity. As two major subcortical projections to the NAc shell, the amygdala and hippocampus are necessary for selecting advantageous and efficient actions. Study of the characteristics of these pathways may reveal differences in connectivity that underscore the complex functions attributed to the NAc shell in normal function and in dysfunction in psychiatric diseases.

Disclosures: L. Marshall: None. **H. Barbas:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.06

Topic: H.06. Social Cognition

Support: CAPES
CNPq
FAPEMIG

Title: Bdnf in the granular layer of the olfactory bulb is recruited for late consolidation of social memory

Authors: *C. M. DE CASTRO, MS, A. S. ALMEIDA, G. S. P. MORAES;
Physiol. and Biophysics, Univ. Federal de Minas Gerais, Belo Horizonte, Brazil

Abstract: Brain-derived neurotrophic factor (BDNF) stands out for being an important modulator of cognitive processes. Previous studies show that BDNF plays an important temporal role for spatial and fear memory in the hippocampus. However, its role in social memory (SM) where the olfactory bulb (OB) is a relevant substrate remains unknown. In the present study, we tested the hypothesis that BDNF intra-OB in different zeitgeber (ZT) is essential for late consolidation of SM. 210 adults (8-12 weeks of age) and 32 juveniles (21-30 days of age, only used as a social stimulus) ICR-CD1 male mice were used. All experiments were performed in compliance with the guidelines from the National Council for Animal Experimentation Control (CONCEA-BRAZIL) and protocols were approved by the Institutional Ethics Committee on the Use of Animals at the Universidade Federal de Minas Gerais (no. 219/2018). SM was tested using the social recognition task and pharmacological tests were performed using guide cannulas positioned in the OB by stereotaxic surgery. Grouped animals received bilaterally 0.5 μL/side of anti-BDNF antibody (1 ug/μL) and isolated animals 0.5 μL/side of human recombinant BDNF (hrBDNF) (0.5 μg/μL) in ZT13 or ZT1. Immunoblotting was used to quantify total BDNF expression and immunofluorescence to quantify BDNF expression per layer of the OB. First, we demonstrate that BDNF isoforms expression does not change between ZT's 1, 4, 7, 13 and 19. Then, intra-OB blockade of BDNF in ZT13 or ZT1 after 12h of training impaired the SM. Nevertheless, intra-OB administration of hrBDNF in ZT13 or ZT1 after 12h of training in isolated animals (SM deficit model) did not reverse SM impairment. Finally, we demonstrate that BDNF expression decreases in the granular layer of the OB after 12h (ZT13) of training. In summary, these results suggest that BDNF in the granular layer of the OB is recruited to modulate some internal process in this substrate for SM to be consolidated. Furthermore, it was possible to demonstrate that memory impairment caused by isolation is not associated with alterations of BDNF levels in late consolidation.

Disclosures: C.M. De Castro: None. **A.S. Almeida:** None. **G.S.P. Moraes:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.07

Topic: H.06. Social Cognition

Support: NSERC

Title: Estrogens in the medial Prefrontal Cortex of ovariectomized female mice rapidly facilitate social recognition but not object recognition or object placement

Authors: *S. PENG, O. KACHMARCHUK, M. WILSON, E. CHOLERIS;
Univ. of Guelph, Univ. of Guelph, Guelph, ON, Canada

Abstract: Estrogen, in the form of 17β-estradiol (E2), rapidly facilitate short-term memories of various social and non-social tasks in mice, when infused into the dorsal hippocampus, a brain region critical for memory formation. Medial Prefrontal Cortex (mPFC) receives extensive dorsal hippocampal projections[EC1] and has high estrogen receptors expression. However, whether E2 in mPFC rapidly facilitate short-term memory remains unclear. In this study, adult ovariectomized female mice were infused bilaterally into mPFC with either vehicle or one of the three doses (25, 50, 100nM) of E2, then tested 15-min post-infusion in either of social recognition (SR), object recognition (OR), or object placement (OP) short-term memory tasks, all in 'difficult' version, in which control ovariectomized mice]show no short-term memory. Results show that E2 in mPFC rapidly facilitated SR but not OR or OP short-term memory, suggesting a possible prioritization of E2's rapid action in mPFC, towards social cognition. Therefore, one additional social cognitive task, the social transmission of food preferences (STFP), is currently being tested. Altogether, this study helps elucidate estrogens' role on rapid short-term memory facilitation in mPFC of female mice and determine whether, differently from the dorsal hippocampus, in the mPFC they preferentially facilitate social over non-social cognition. Funding: This study was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).(* Equally contributed to this work)

Disclosures: S. Peng: None. **O. Kachmarchuk:** None. **M. Wilson:** None. **E. Choleris:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.08

Topic: H.06. Social Cognition

Support: McGovern Institute of Brain Research
Mathworks Fellowship
Simons Center for the Social Brain
Dr. Michael Stiefel

Title: Causal inference using the experiences of self and others

Authors: *S. RADKANI[1,2], S. M. YOO[1,2], M. JAZAYERI[1,2];
[1]Brain and Cognitive Sci., [2]McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: In social contexts, humans and animals infer latent causes from the experiences of others. However, the computational and neural mechanisms of social inference remain elusive. Here, we report our initial findings in a project aimed at understanding how humans and monkeys infer the latent states of the environment based on their own experience (self-learning) as well as experiences of others (observation-learning). We designed a novel foraging task with two levels of hierarchy. First, players had to choose between two foraging sites and then had to control an avatar with a joystick to intercept passing-by tokens on a display. Importantly, the site with the reward switched covertly after a random number of trials and reward delivery was stochastic with a probability that increased with the number of intercepted tokens. This causal structure made it necessary for players to (1) track the history of decisions, performances, and outcomes to infer the rewarding site, and (2) try to intercept as many of the tokens as possible. Starting with a single-player version, we verified that humans (N=10) and monkeys (N=2) integrate information across multiple trials to update their belief about the rewarding site. Next, we had the same subjects play the two-player version in conspecific pairs. In this version, both players reported their preferred site but only one randomly selected player proceeded to collect tokens in their chosen site ('actor') while the other ('observer') watched the actor play. Importantly, the observer had visual access to the entire sequence of actions and outcomes making it possible to compare the efficacy of self-learning to observation-learning. We found that, on average, humans discount the outcome brought about by others compared to themselves. This finding reveals an inherent asymmetry between self- and observation-learning even when the external information is perfectly matched between the two modes of learning. Monkeys were also able to learn from one another. However, compared to humans, their behavior showed more sensitivity to outcomes and less sensitivity to decisions and performance of the other animal. They also did not discount observational outcomes as much as humans. We are currently using modeling and electrophysiology to understand the mechanistic factors that lead to discounting social outcomes, and putative species' differences in observation-learning.

Disclosures: S. Radkani: None. **S.M. Yoo:** None. **M. Jazayeri:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.09

Topic: H.06. Social Cognition

Support: J. Douglas Tan Postdoctoral Fellowship

Title: A paradigm for one-shot learning from experience and observation in non-human primates

Authors: *R. CHEN[1,2], M. JAZAYERI[1,2];
[1]McGovern Inst. for Brain Res., [2]Dept. of Brain & Cognitive Sci., MIT, Cambridge, MA

Abstract: Primates can learn what to do and what not to do from a single experience or observation. For example, monkeys can infer dominance status in a new troop from a single interaction. A newcomer can also infer the status from observing aggressive or submissive gestures between members of the troop. How does the brain implement this ability to infer hidden information rapidly and flexibly from experience and observation? Here, we report our initial results in a project aimed at understanding the neural basis of one-short learning from experience and observation. We designed an approach-or-avoid game for macaque monkeys. In this task, the player controls an avatar's movement on the screen with a joystick. Objects with hidden identities (either 'prey' or 'predator') move down from the top of the screen in random order, and the player must decide whether to approach or avoid them. When the avatar contacts a prey, the subject is rewarded with juice; when it touches a predator, the trial ends with no reward and a timeout period. To encourage rapid learning on the time scale of a single trial, we changed the appearance of prey and predator to new combinations every two trials with the first trial providing an opportunity for one-short learning and the second trial serving as the test for such learning. So far, we have tested one animal in this task in two conditions, a self-learning condition and an observational condition involving a computer agent. In the observational condition, a computer agent controls a demonstrator avatar in the first trial before the subject plays for the second trial. In the self-learning condition, the animal exhibited hallmarks of one-shot learning: after a single contact with either a new prey or predator, the animal learned the identity of both objects and acted accordingly. Strikingly, the animal could make inferences by exclusion: after experience of getting reward from a prey, it avoids future predators without having experienced contacting that predator, and vice versa. Surprisingly, this rapid learning was nearly absent while observing a computer agent. As the trajectories were sampled from the animal's own play, this indicates the animal did not learn from the sensory statistics of the visual input alone. A growing body of work indicates that the presence of agency may facilitate learning. Therefore, we hypothesize that one-shot observational learning may be restored if the animal watches a conspecific - not a computer agent - play the approach-or-avoid game. We are currently training a second animal to test this hypothesis.

Disclosures: R. Chen: None. **M. Jazayeri:** None.

Poster

655. Social Cognition: Animal Behavior II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 655.10

Title: WITHDRAWN

Poster

656. Prefrontal Cortex Role in Memory and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 656.01

Topic: H.09. Spatial Navigation

Support: R01 MH113626
F99 NS119001

Title: Neuronal Representations in Medial Prefrontal Cortex during Nonspatial Memory for Sequences of Events

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Abstract: Memory for sequences of events organizes episodic experiences into serial or ordinal associations representing the flow of events as they occurred. It has been established that sequence memory depends on prefrontal-hippocampal interactions. We recently showed that specific cell populations in medial prefrontal cortex (mPFC) differentially contribute to retrieval strategies in sequence memory (Jayachandran et al., 2019, *CellRep*). Yet, how individual mPFC neurons respond while processing sequence memory is not fully understood. Here, rats were trained to remember two sets of four odor sequences (Side 1, Sequence 1: A-B-C-D and Side 2, Sequence 2: W-X-Y-Z) presented at opposite ends of a linear track. Rats demonstrated sequence memory in both sequences by holding their nose in the port for >1s for in sequence odors and withdrawing prior to 1s for out of sequence odors, for a small water reward. We implanted five rats with Neuronexus silicon probes with a 2X4 tetrode arrangement targeting mPFC (prelimbic cortex) and the hippocampus (HC; SLM of dorsal CA1). Overall, we recorded 481 mPFC neurons during the sequence memory task. Single mPFC neurons were isolated offline, and their spike times analyzed across task variables (odors, positions, sequential context, and accuracy) in temporal analysis windows aligned to poking behaviors (poke in/out). A firing rate criterion of greater than 1 Hz within the poke out event and the removal of multi-unit properties led to a final count of 231 single units. Results showed that ~20% of mPFC neurons were related to position, sequential context, and/or accuracy, but not odors. Moreover, mPFC spike activity changed around poking behaviors with ramping-like firing patterns. These response patterns generalized between sequences and were thus nonspatial. Moreover, three distinct cell clusters were identified via k-means algorithms using the spike properties of width, firing rate, and valley-to-peak slope. Ensembles were separated based on when they were active during a trial (before, during, or after poke out). These separate cell populations provided different information about position, sequence, and accuracy. Spike-phase relationships in mPFC spikes were found to be entrained to local delta around poking behaviors and entrained to beta and theta in relation to trial accuracy. These spiking dynamics raise interesting questions about the supremacy of different rhythmic mechanisms governing separate mPFC populations during sequence memory.

Disclosures: S. Leyva: None. **A. Vasallo Veliz:** None. **M. Jayachandran:** None. **R.P. Vertes:** None. **T.A. Allen:** None.

Poster

656. Prefrontal Cortex Role in Memory and Fear

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Topic: H.09. Spatial Navigation

Support: NIH MH113626
FIU CASE Distinguished Postdoctoral Program

Title: Optogenetic stimulation of nucleus reuniens of the thalamus elicits beta coherence in the medial prefrontal-hippocampus network

Authors: *T. D. VIENA[1], B. SETLOW[2], F. D. LUBIN[3], R. P. VERTES[4], T. A. ALLEN[1]; [1]Florida Intl. Univ., Florida Intl. Univ., Miami, FL; [2]Univ. of Florida, Univ. of Florida, Gainesville, FL; [3]Univ. Alabama Birmingham, Univ. Alabama Birmingham, Fultondale, AL; [4]FAU/Ctr Complex Systems, Florida Atlantic Univ., Boca Raton, FL

Abstract: Abnormal synchronization in the prefrontal-hippocampal circuit is a hallmark of several neuropsychiatric disorders. Anatomically, nucleus reuniens (RE) projects to both the medial PFC (mPFC) and hippocampus (HC), including neurons that project to both structures (Viena et al., 2021, *Hippocampus*). RE is involved in cognitive tasks that require mPFC-HC interactions and in the rhythmic activity of the mPFC-HC system in the theta (6-12Hz) and delta bands (1-4Hz; Dolleman-van der Weel et al., 2019, *LearnMem*). One current hypothesis is that RE is responsible, in part, for mediating coordinated interactions within the mPFC-HC network in support of cognition. It is unclear, however, which mPFC-HC rhythms are modulated by RE neurons. We tested this capacity using an optogenetics/AAV-retrograde strategy to differentially activate RE neurons projections to ventral CA1, a region innervated by RE. Rats received retrograde AAV injections of channelrhodopsin (ChR2) in vCA1(n=4), while control rats received non-ChR2 AAV (n=5). All rats were implanted with stainless-steel wire electrodes in mPFC and vCA1 to record local-field potentials (LFP). An optrode above RE activated ChR2-expressing cells with blue light. Recordings occurred in an open field during free behavior. ChR2-expressing RE neurons were stimulated with square pulses or sinewaves at frequencies known to be intrinsic to RE neurons (Viena et al., 2021, *BehavBrainRes*; 5 min blocks each). We observed that pulses evoked strong CA1 monosynaptic responses (~12 ms latency) consistent with the glutamatergic actions of RE in CA1, as previously described. Second, when stimulated at the various frequencies and waveforms, the resultant LFP rhythm included the stimulated frequency superimposed on the intrinsic activity of experimental rats but not in controls. Notably, we observed a predominant beta rhythm during stimulation, regardless of the stimulus frequency, which persisted after the cessation of stimulations. When we assessed coherence during and 1 min after the end of stimulations, theta coherence was reduced, but beta coherence increased in experimental rats, but not in controls. Taken together these results demonstrate that RE activation drives beta coherence in the mPFC-HC network regardless of the input. Interestingly, this pattern of decreased theta and increased beta coherence in mPFC-HC has also

been described by our lab during the nonspatial sequence memory task (Jayachandran* and Viena* et al., 2022, *bioRxiv*). Our findings support the idea that RE influences the activity of mPFC and HC in the theta and beta ranges to support the cognitive processes associated with mPFC-HC interactions.

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Poster

656. Prefrontal Cortex Role in Memory and Fear

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Topic: H.09. Spatial Navigation

Support: R01 MH113626
F99 NS119001

Title: Reuniens synchronizes with medial prefrontal cortex and hippocampus at beta frequencies during nonspatial memory for sequences of events in rats

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Abstract: Episodic-like memory is adaptive in its ability to advantage future behaviors, and depends on the interactions of the medial prefrontal cortex (mPFC) and the hippocampus (HC). A general axiom is that mPFC exercises executive control over memories that are otherwise acquired and represented by HC (e.g., Jayachandran et al, 2019, *Cell Reports*). While HC projects to mPFC, only sparse return projections have been identified. Instead, interactions depend on the nucleus reuniens (RE), a midline thalamic region that provides bi-directional control over mPFC-HC loops. The most prevalent theory of RE in memory is that it helps control mPFC-HC interactions by driving changes in the network-wide oscillatory synchronous states. But if and when mPFC-HC networks synchronize during memory, and how this is related to RE, remains poorly understood. To look at this, we tested two groups of rats on a sequence memory task with two sequences located in nose ports at opposite ends of a linear maze. Rats demonstrated sequence memory by holding for >1s for in sequence odors or withdrawing prior to 1s for out of sequence odors to receive a small water reward. The first group of rats (n=5, 3 females) were implanted with silicon probes targeting mPFC (prelimbic cortex, layer VI) and HC (dCA1) and the second group of rats (n=4, 3 females) were implanted with silicon probes targeting RE and HC (dCA1). It has been shown that CA1 engages memory-related beta (15-30Hz) during sequence memory reflecting memory and sequential contexts (Gattas et al., 2022, *eLife*, Allen et al., 2016, *JNeurosci*). Here we analyzed LFPs from mPFC, RE and HC using

perievent power spectral densities and by looking at coherence between mPFC-HC and RE-HC. We observed memory-related mPFC-HC beta coherence as the dominant rhythm on memory trials, while theta dominated running bouts. Beta bursts onset ~400ms after trial initiation in mPFC and HC. Beta peaked before decisions were executed, and reflected the sequential contexts. In the RE-HC group, we also observed memory-related beta bursts in RE, which occurred ~75ms earlier during individual trials than in mPFC-HC, and lasted until after decision, suggesting RE might mediate mPFC-HC beta. We also found that RE beta amplitudes parametrically decline across sequential positions potentially reflecting changing working memory demand as the rat progressed through a sequence, consistent with cortical beta reported in primate studies. Taken together, these experiments demonstrate beta as a key rhythm synchronizing a large mPFC-RE-HC networks in support of successful memory-based decision making.

Disclosures: M. Jayachandran: None. **A. Garcia Barrabeitg:** None. **R.P. Vertes:** None. **T.A. Allen:** None.

Poster

656. Prefrontal Cortex Role in Memory and Fear

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Program #/Poster #: 656.04

Topic: H.09. Spatial Navigation

Support: MH113626

Title: Role of the reuniens-corticohippocampal circuit in flexible use of temporal strategies for elapsed time memory

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Abstract: Anatomical and functional coupling of the hippocampus (HF) and the medial prefrontal cortex (mPFC) is central to the construction of episodic memories. While episodic memories are composed of interrelating topographies of the ‘what’ and ‘where,’ it can be fundamentally disambiguated by ‘when’ an event occurred. A vital constituent of this is elapsed time memory, or memory of how long ago a particular event occurred. Elapsed time at longer time scales is represented in the HF (Kesner and Hunsaker, 2010; Jacobs et al., 2013; Palombo et al., 2016), which in turn engages with the mPFC to select appropriate behavior/outcomes. In the

rat, the ventral hippocampus (vHF) innervates the mPFC, but is met with limited mPFC-HF return projections. With virtually no dorsal hippocampal (dHF) efferents to the mPFC, most mPFC-HF communication relies on polysynaptic circuitry. Regarding this, the thalamic nucleus reuniens (RE) is positioned as a key intermediary completing mPFC-HF loops. Recent studies have highlighted the integrity of mPFC-RE-HF circuitry in spatial, temporal, contextual and affectual components of memory (Dolleman-van der Weel et al., 2019). Here we examined whether RE, via its projections to the mPFC/HF, is recruited in elapsed time memory using a spatiotemporal paradigm adapted from Jacobs et al. (2013). Long Evans male rats (n=9) were trained on response contingencies which paired a directional response in a T-maze with elapsed time intervals (1 vs 12 min) separated by large differences. Rats were then microinjected with the hM4Di DREADD (AAV9.CAG.mCherry-2a-hM4dnrxn.WPRE.SV40) into RE and implanted with bilateral indwelling cannula targeting the mPFC and intermediate vHF. CNO-induced inhibition of RE or RE terminals did not impair the ability to discriminate between 1 vs.12 min when compared to vehicle infusions. This indicated that quelling RE activity had no effect on the ability of rats to acquire the spatial, associative and motivational demands of the task. Rats were next trained to discriminate between intervals with small temporal differences (8 vs. 12 min), requiring a shift in their directional response from 1 to 8 minutes. Here, CNO suppression of RE activity and RE terminals to the mPFC and HF reduced mean accuracy across sessions. Notably, rats exhibited biased temporal responses towards previously learned (12 min) trials following inhibition of RE->PFC terminals. Collectively, these results uncovered a thalamocortical circuit for fine, but not coarse, discriminations of elapsed time memory while highlighting a role for the RE->PFC pathway in temporal cognitive strategies driving flexible goal directed behavior.

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Poster

656. Prefrontal Cortex Role in Memory and Fear

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FIU Undergraduate to Graduate Program Fellowship

Title: The thalamic nucleus reuniens changes prefrontal-hippocampal rhythmic modes dependent on behavioral states in the rat

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[1]Psychology, [2]Dept. of Psychology, Florida Intl. Univ., Miami, FL

Abstract: The nucleus reuniens of the thalamus (RE) mediates interactions between the medial prefrontal cortex (mPFC) and hippocampus (HC), and is critical to several forms of memory (e.g., Dolleman-van der Weel et al. 2019, *LearnMem*). One prominent theory of RE is that it helps memory by mediating the rhythmic synchrony between mPFC and HC during directed encoding and retrievals, and/or working memory (Jayachandran et al., 2019, CellRep; Viena et al., 2018, Hippocampus). It is known that running and stationary behaviors correspond to distinct rhythmic modes in the brain, including a prominent running-speed modulated theta rhythm (6-12Hz) and stationary-related 'awake' delta rhythm (1-4Hz) in both the mPFC and HC (Schultheiss et al., 2020, *BehavNeuro*). However, the relationship between these basic behaviors and their corresponding oscillatory rhythms in the mPFC-RE-HC circuit are poorly understood. Here, we used DeepLabCut (a machine-learning based behavioral tracking algorithm) in combination with multisite electrophysiological recordings of local field potential (LFP) activity in mPFC and ventral HC during optogenetic stimulations in RE neurons projecting to vHC (retroAAV-ChR2) in freely behaving rats in an open field maze. We separated running bouts (running > 5cm/s with a peak speed of 15cm/s) and stationary periods (<5cm/s) during RE stimulations. Bouts had a minimum duration of 2.05s, and body direction changes beyond 52 degrees and head angle with respect to the center of the body above 35 degrees (for running only) were excluded. We compared control and experimental rats' running and stationary states and found a significant increase in stationary bouts during RE stimulations ($p < .001$). Next, we assessed mPFC-HC coherence (1-50Hz) sorted by these behavioral states. We found that during stationary periods theta was not changed by RE stimulations, but by contrast delta and beta (15-30Hz) significantly increased (p's < .05). During running periods, theta and delta were statistically unchanged, but beta was significantly increased ($p < 0.01$). Interestingly, we found a significant interaction effect of RE stimulation x behavior on mPFC-HC beta coherence ($F_{(1,16)}=5.757$, $p< .05$) such that RE-induced beta is enhanced during stationary behavior suggesting the ability of RE to drive synchrony is dependent on the current behavioral state. Notably, this increase in RE-induced beta coherence in mPFC-HC sites is remarkably consistent with other findings in our lab that demonstrate that beta coherence in the mPFC-RE-HC system increases during a nonspatial sequence memory task (Jayachandran* and Viena* et al. 2022, *bioRxiv*).

Disclosures: V. Roldan: None. **T.D. Viena:** None. **T.A. Allen:** None.

Poster

656. Prefrontal Cortex Role in Memory and Fear

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European Research Council ('NavigationCircuits' GA714642)

Title: The prefrontal-reuniens-hippocampal circuit supports planning of navigational routes

Authors: *H.-A. KIM[1], S.-F. YEN[3], H. T. ITO[2];
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Abstract: Goal-directed spatial navigation requires accurate estimates of an animal's position and its destination, and these estimated positions are further needed for planning of a goal-directed path avoiding known obstacles in the environment. However, the circuit mechanism underlying the animal's route planning ability is still largely unclear. Previous studies described that neurons in the hippocampus (HPC) encode an animal's own position, whereas those in the orbitofrontal cortex - a subregion of the prefrontal cortex (PFC) - represent its goal destination during navigation (Basu et al., 2021). We thus hypothesize that interactions of two map systems in PFC and HPC are essential for planning of goal-directed paths and this PFC-to-HPC communication is mediated by the pathway including the thalamic nucleus reuniens (RE) as a relay (Vertes et al., 2007; Ito et al., 2015). To assess the animal's route planning ability, we used a modified version of the task developed by Pfeiffer & Foster (2013), in which an animal is required to navigate to a fixed goal location from different starting positions in an open-field arena. Unlike the original task, we added a wall inside, imposing an animal with an additional demand for planning of a wall-avoiding route. We confirmed that rats can memorize the wall location and take a smooth wall-avoiding route even in darkness. To perturb the PFC-RE pathway, we injected a virus expressing the excitatory opsin bReaChES in PFC neurons to excite their axon terminals in RE. We found that perturbations of the PFC-RE pathway caused the animal to change its moving direction frequently during a goal-directed journey, resulting in an inefficient longer path. By contrast, when the goal could be approached by a straight path from the starting location, we did not observe any behavioral deficit under the same manipulation, indicating a selective role of the PFC-RE pathway in planning of a wall-avoiding route. To assess the impact of RE inputs on HPC, we recorded the activity of neurons in the hippocampal CA1 of the animal expressing the inhibitory opsin SwiChR++ in RE. By employing a Bayesian decoding approach before the onset of navigation, we confirmed that CA1 neural ensembles generated brief sequential firing representing trajectories corresponding to the upcoming goal-directed journey avoiding the wall location. However, these wall-avoiding trajectory sequences were largely diminished by the silencing of RE neurons. Our results together point to the PFC-RE-HPC pathway as a key circuit element supporting planning of goal-directed navigational routes, and this process is likely through interactions of the two map systems in PFC and HPC.

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Poster

656. Prefrontal Cortex Role in Memory and Fear

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NSF Grant IOS 175111

Title: Perirhinal-prefrontal cortical connections support cued-elicited expression of conditioned fear responses.

Authors: N. A. VANG, R. A. K. PUREWAL, D. GONZALEZ MAGANA, *S. C. FURTAK; Psychology, Sacramento State, Sacramento, CA

Abstract: As a major input structure to the hippocampal memory system, the perirhinal cortex (PER) supplies stimulus and object information to the hippocampus. This function is supported by unimodal and polymodal sensory information the PER receives from cortical and subcortical afferents, making the PER well positioned to associate and process stimulus information. Previous research demonstrates that the PER has a pivotal role in visual memory tasks such as object recognition and delay non-match to sample tasks. More recently, the PER has been implicated in fear learning tasks particularly when the stimuli representation require unitization across modality, features, or time. In the current study, we assess whether the connection between the PER and the medial prefrontal cortex (mPFC) is important for fear expression or extinction to a stimulus that require unitization across time (a discontinuous light conditioned stimulus, CS). Male Spraque-Dawley rats (310-452g) were assigned to one of three groups based on whether they received a PER-mPFC cross-lesion or a unilateral lesion to either the PER or the mPFC. On Day 1, Fear Acquisition, animals received 5 co-terminating presentations of the CS paired with a brief foot shock (unconditioned stimulus, US). Three days later rats underwent lesion surgery and then were allowed to recover. On the tenth day after surgery, Extinction Training, rats were returned to the conditioning chamber and received 20 CS-alone presentations. On the next day, Extinction Retrieval, rats once again returned to the conditioning chamber and received 15 CS-alone presentations. Rats in the cross-lesion group showed significant impairments in fear expression, indicated by significantly reduced freezing during both Extinction Training and Extinction Retrieval compared to both unilateral lesion groups. This study supports a growing body of research highlighting the PER as part of a wider system of structures engaged in cue-elicited fear learning.

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Poster

656. Prefrontal Cortex Role in Memory and Fear

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NRF-2022R1A2C2009265

Title: Amygdala-prefrontal cortex interactions during risky decision-making in rats encountering a predatory threat in a naturalistic environment

Authors: *J. J. KIM[1], E. KIM[1], S. PARK[2], J.-S. CHOI[3], J. CHO[2];
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Abstract: Animals often change their foraging behaviors under predation pressure to maximize survival by decreasing exposure to threats and increasing procurement of available food. Previously, we investigated foraging behaviors in an ecologically-relevant setting and found that rats can discern conditional predatory (Robogator) threats and adjust their foraging strategy, which was abolished by amygdala lesions (Kim et al., 2016). Here, we explored the neural basis of conditional threat discrimination and foraging preference switch by simultaneously recording single-units in the basolateral amygdala (BLA) and the prelimbic (PL) area of the medial prefrontal cortex, two structures implicated in fear and decision-making processes, respectively (Mobbs and Kim, 2015). Male Long-Evans rats were implanted with tetrode arrays in the BLA and PL and underwent successive stages of (i) nest habituation, (ii) foraging preference baseline (choosing chocolate pellet vs. normal pellet), and (iii) Robogator encounter testing (preference baseline trials followed by Robogator trials). Neural activities of BLA and PL were recorded simultaneously during the Robogator encounter testing days. During the preference baseline trials, rats showed a general bias towards chocolate pellets over normal pellets. Upon approaching the preferred pellet and experiencing the Robogator surge, all animals shifted their foraging behaviors toward non-preferred pellets. When BLA and PL cells were analyzed by plotting the peri-event time histograms, the population levels of BLA neurons showed increased activity exclusively to the Robogator surge while the population levels of PL neurons displayed increased activity comparably to multiple events (i.e., preferred pellet, non-preferred pellet, and Robogator surge). A cross-correlation analysis revealed that a subset of simultaneously recorded BLA and PL cell pairs showed increased spike synchrony prior to foraging preference shift during the Robogator session, where (PL-led) BLA cells responded to the Robogator surge while (BLA-projecting) PL cells responded to multiple events. These results suggest that BLA neurons encoding imminent threats actively interact with PL units signaling multiple events to change foraging strategy under conditional threat.

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Poster

656. Prefrontal Cortex Role in Memory and Fear

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NSF Grant IOS 175111

Title: Chemogenetic silencing of the perirhinal cortex impairs retrieval of the extinction memory to a discontinuous conditioned stimulus.

Authors: *J. H. LAMB, E. P. DUKA, P. DEMYANCHUK, S. C. FURTAK;
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Abstract: Within the medial temporal lobe memory system, the role of perirhinal cortex (PER) in memory formation versus perceptual processing has been debated for decades. While one dominant theory suggests that the main function of the PER is object recognition memory, other theories have proposed that the PER processes and represents stimuli that require unitization across features, modality or time. In support of the latter theory, an increasing number of studies have indicated that the PER supports fear conditioning to stimuli that require unitization across modalities or time. A recent publication using muscimol inactivation of the PER via cannulation suggests that PER involvement in fear learning extends to fear extinction of discontinuous conditioned stimuli (CSs). Here, the current study builds on this line of research by silencing the PER with designer receptors exclusively activated by designer drugs (DREADDs), which allows for a broader inactivation of perirhinal neurons along its rostrocaudal extent compared to traditional cannulation techniques. Male Sprague-Dawley rats underwent surgeries that target PER bilaterally with an adeno-associated virus (AAV) carrying a modified form of the human inhibitory muscarinic 4 receptor (hM4Gi) that is activated by clozapine-N-oxide (CNO). Three weeks after surgery, rats began a three-day fear extinction paradigm. On day 1, Fear Acquisition, rats received 5 presentations of a discontinuous light CS that co-terminated with a brief foot shock (unconditioned stimulus, US) in a conditioning chamber. On day 2, Extinction Training, rats received intraperitoneal injections of either CNO (Experimental group) or vehicle (dimethyl sulfoxide, DMSO; Control group). After 45 mins, rats were returned to the conditioning chamber and received 20 CS-alone presentations. On day 3, Extinction Retrieval, rats were again returned to the same context and received 15 CS-alone presentations. Cue-elicited freezing levels were significantly elevated in the Experimental group compared with the Control group during both Extinction Training and Extinction Retrieval. These results indicate that while rats are able to retrieve the original fear memory, there were significant impairments in both within session extinction learning and retrieval of the extinction memory. This study provides further support that the PER plays an important role in fear extinction when the stimulus requires unitization across time.

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Poster

656. Prefrontal Cortex Role in Memory and Fear

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Topic: G.01. Fear and Aversive Learning and Memory

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Title: Ecological analysis of Pavlovian fear conditioning in rats

Authors: *P. R. ZAMBETTI[1], B. P. SCHUESSLER[2], B. E. LECAMP[3], A. SHIN[4], E. KIM[1], J. J. KIM[1];
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Abstract: Contemporary models of fear and their putative translational significance largely stem from rodent Pavlovian fear conditioning research, which simplifies behavioral, systems, circuit and genetic analyses of the acquisition, expression, generalization, extinction and return of a specific conditioned fear responses (CR; mainly, freezing). Although fear conditioning is widely considered crucial to survival, its functions surprisingly have not yet been ethologically validated. Some have even questioned its evolutionary relevance—if associative trial-and-error learning were the primary defensive mechanism, most animals would be killed before they learned which predators and situations must be avoided (e.g., Bolles, 1970). To address this critical gap in proof of concept, we incorporated a one-trial delay auditory fear conditioning procedure into an ethologically-relevant 'approach food-avoid predator' scenario. Specifically, male and female Long-Evans rats foraging for food in a large arena were presented with a tone conditioned stimulus (CS) paired with electric shock unconditioned stimulus (US) to their dorsal neck/body that reflexively elicited escape unconditioned response (UR) to the safe nest. On subsequent test days, the tone-shock paired animals failed to exhibit fear CR (neither fleeing nor freezing) to the CS introduced in the open arena. The same tone CS-shock US arrangement in a standard conditioning chamber, however, produced a robust freezing CR to the CS. To mimic a more naturalistic fearful scenario, other animals encountered a looming artificial owl paired with a dorsal neck/body shock, mimicking a realistic predatory-inflicted pain. The owl-shock paired animals instantly fled to the nest when presented with a tone for the first time. These results highlight the possibility of a nonassociative, rather than standard associative, fear process providing adaptive function in life-threatening situations that animals are likely to encounter in nature. The utilization of naturalistic fear paradigms that simulate dangers that animals and humans encounter in real life—where there are external hostile agents (predators in animals and perpetrators in humans)—will likely clarify, update, and revise fear concepts derived largely from fear conditioning studies (devoid of external agents) and in doing so facilitate future progress in the treatment of fear disorders.

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Poster

657. Human Learning and Memory

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Title: Where do I remember this? Recognition memory for low-level visual stimuli.

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Abstract: Most theories of memory assume that long-term, declarative memory relies primarily on the medial temporal lobes (MTL). In contrast, the Representational-Hierarchical (RH) Account - challenges this claim, replacing it with two assumptions: (1) the brain contains a hierarchy of stimulus representations, from simple features (lines, blobs) in visual cortex to high-dimensional associative representations in MTL; and (2) any region in the hierarchy can support memory for the information that it represents. The RH Account thus predicts that long-term, declarative memory for simple, purely visual stimuli should recruit regions outside of the MTL, specifically, in visual cortex. In this study, participants studied a set of simple visual stimuli for 10-20 days via visual search training. Stimuli were built from conjunctions of shape and fill pattern. At test in the MR scanner, subjects saw Studied items, Recombination items (novel recombinations of features of studied items), and Novel items. Participants responded “Old”, “Recombo” or “New” with a button press. Average memory performance (i.e., accuracy) showed that subjects are capable of distinguishing studied visual stimuli (i.e. Old) from novel stimuli (i.e., Recombo, and New). Multivariate classifier analysis (i.e., MVPA) of fMRI brain patterns was used to investigate which brain areas drive successful identification of studied stimuli. Specifically, we asked which regions hold neural representations that permit recognition memory for visual conjunctions and for visual features. Using signal detection theory, classifier prediction results were analyzed by looking at the discriminability of Old and Recombo items (a measure of conjunction memory), against the discriminability of Old and New items (a measure of feature memory). Results suggest that early visual regions (V1-V3) hold memory representations most apt for recognition of low-level features. Meanwhile, more anterior regions, such as lateral occipital (LO) cortex and areas in parietal cortex (i.e., IPS, SPL) seem to hold representations of memory for low-level visual conjunctions, necessary for discriminating Old and Recombo stimuli. In conclusion, results imply that “sensory” brain regions, posterior to MTL, are supporting long-term memory behavior by holding neural representations of visual conjunctions and visual features needed for the memory task.

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Poster

657. Human Learning and Memory

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Program #/Poster #: 657.02

Topic: H.07. Long-Term Memory

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American Academy of Sleep Medicine Strategic Research Award

Title: Greater resting-state functional network integration supports successful mnemonic discrimination following sleep

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Abstract: The functional connectome of the human brain exhibits modular organization. In the context of memory consolidation, reactivation of a memory trace during sleep involves reinstatement of a hippocampal index. This reinstates patterns of activity stored in cortical modules which, over time, become interconnected to promote consolidation. Surprisingly, graph theory has been rarely applied to examine the functional role of modularity in memory consolidation. We examined whether network modularity (Q), an index of module segregation, is associated with emotional memory consolidation and sleep architecture in seventeen healthy older adults (μ=73.1±5, 9F). Participants completed overnight sleep assessment with high-density electroencephalography and polysomnography and performed the emotional version of the Mnemonic Discrimination Task, which assesses the ability to discriminate among similar negative, neutral, and positive images—prior to and following overnight sleep. The Lure Discrimination Index (LDI) measures this discrimination ability and was calculated before and after sleep. Structural and resting-state fMRI data were collected using a 3T Siemens Magnetom Prisma Scanner. All neuroimaging data were preprocessed with the CONN toolbox. The Brainnetome Atlas was used to define network nodes and weighted signed adjacency matrices were derived. Q was computed using the Brain Connectivity Toolbox by running 150 iterations of the Louvain Modularity Maximization algorithm to generate consensus partitions across four different resolution parameters. Preliminary results indicated that discrimination of positive stimuli was preserved following overnight sleep, whereas performance for negative and neutral stimuli significantly deteriorated. We observed that lower Q, indicative of reduced modularity or greater network integration, was associated with better discrimination of low similarity stimuli—most strongly with those of positive valence—following sleep. Lower Q was also associated with less time spent in REM sleep. These findings may suggest that greater network integration

supports successful mnemonic discrimination of positive information following overnight sleep, which may be related to changes in REM sleep expression. Decreased network modularity and related REM sleep reductions may be potential mechanisms facilitating the positive memory bias reported in older adulthood.

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Poster

657. Human Learning and Memory

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Topic: H.07. Long-Term Memory

Support: UK Medical Research Council G03000117
UK Medical Research Council G1002276

Title: Amygdala structure and function is altered in patients with developmental amnesia

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Abstract: A number of pathological events, in particular during early life, can lead to hippocampal injury, but the impact of such damage on the development of the interconnected amygdala has not been investigated as yet in human patients. Here, we used manual segmentation on MRI acquisitions to estimate the volume of the amygdala in a large cohort of twenty-three patients with developmental amnesia (DA), a syndrome associated with early-life hypoxia-induced bilateral hippocampal atrophy and selective episodic memory impairment. Our estimates revealed that in our group of controls, amygdala volume correlated with hippocampal volume in both hemispheres. In patients with DA, the amygdala was 12% smaller than in controls ($p < .001$) and this atrophy was more severe in the left (-17%, $p < .001$) than in the right hemisphere (-6%, non-significant). In the DA group, amygdala volume correlated with hippocampal volume in the left hemisphere only. Volume estimates were analyzed in relation to scores obtained on a parental questionnaire (Child Behavior Checklist). Increased socioemotional and/or behavioral problems in the domains of anxiety and stress, in patients with DA relative to controls, were significantly correlated with left amygdala volume. Two patients with DA presenting with mild to severe amygdala atrophy were tested for facial emotion recognition. Although eye-tracking analyses revealed that the two patients made use of relevant information to identify emotions, they nevertheless showed deficits, in particular in recognizing “fear”, ”happiness” and “surprise”, as compared to a group of controls. These two patients with

DA also showed an increased latency to respond. Abnormal amygdala development in patients with DA could potentially account for these symptoms, and could be attributable to the indirect consequences of the early hippocampal damage, and the abnormal interaction of the damaged hippocampus with the amygdala during development.

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Poster

657. Human Learning and Memory

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Program #/Poster #: 657.04

Topic: H.07. Long-Term Memory

Support: NIH-AG053555

Title: Cerebellar-cortical functional connectivity and mnemonic discrimination in preclinical Alzheimer's disease

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Abstract: Emerging evidence suggests that functional connectivity (FC) among distributed neural networks is disrupted in preclinical Alzheimer's disease (AD), often preceding accumulation of pathology, and ultimately contributing to cognitive impairment in AD. The cerebellum has long been thought to play a crucial role in motor control, yet recent evidence points to its additional role in nonmotor domains (e.g., memory, executive function, emotion). Neuroimaging studies have also delineated intrinsic cerebellar-cortical functional networks that presumably support cognitive functions, such as the default mode network. Despite the accumulating literature on cerebellar-cortical connectivity, how these networks are altered with AD pathology or how they predict cognitive performance in the early stages of AD remains to be explored. We analyzed data from 22 cognitively unimpaired older adults (age 60 - 86 yrs, 13 females), who completed resting-state fMRI, florbetapir PET to assess amyloid load, as well as the Mnemonic Discrimination Task (MDT), a memory test that is sensitive to hippocampal pattern separation. Seed-based connectivity metrics were computed in the CONN Toolbox using the 7 cerebellar parcellations (Buckner et al., 2011) that are functionally coupled to specific cerebral networks (Yeo et al., 2011). To examine whether the FC patterns are different by amyloid status, participants were stratified into low (n = 12) and high (n = 10) amyloid burden subgroups based on the threshold of global SUVR = 1.11. Individuals with high amyloid burden showed weaker cerebellar-cortical FC across various cortical regions, particularly the somatomotor network. MDT performance was positively correlated with cerebellar-cortical FC in a wide range of networks, including the visual network, somatomotor network, and

frontoparietal network. Our findings suggest that changes in FC between the cerebellum and cortex could be a novel biomarker for preclinical AD and contribute to memory decline.

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Poster

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Program #/Poster #: 657.05

Topic: H.07. Long-Term Memory

Support: NIA AG053555(PI: Yassa)

Title: Cerebrovascular reactivity in the hippocampus predicts mnemonic discrimination performance

Authors: *C. CHWIESKO[1], S. KIM[1], J. ADAMS[1], B. RIZVI[1], P. LIU[2], L. MCMILLAN[1], M. YASSA[1];
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Abstract: The integrity of the brain's vasculature is key to healthy brain function. Alterations to cerebrovascular integrity is suggested to be a factor contributing to disease onset and progression in neurodegenerative disease like Alzheimer's disease. Cerebrovascular reactivity (CVR) - the ability of the brain vasculature to dynamically regulate blood supply - is a more specific indicator of cerebrovascular health compared to other vascular measures like cerebral blood flow or cerebral blood volume. CVR has been found to be diminished in early Alzheimer's disease and shown to be impaired in the hippocampus of young, non-demented APOE4 carriers. In this study, we investigated hippocampal CVR in 21 healthy, nondemented older adults (mean age 68.9 y, 12 F) and examined links between hippocampal CVR and their performance on a Mnemonic Discrimination Task - a task highly sensitive to hippocampal pattern separation. We used a novel CVR mapping approach that allowed us to measure CVR in resting state BOLD fMRI. By exploiting the natural variation in BOLD signal induced by respiration, this method does not require the use of an intricate hypercapnic gas inhalation or breath-holding. We measured CVR in the left and right hippocampus and calculated the correlation with memory performance in the Mnemonic Discrimination Task. We found that the left hippocampus had a significantly higher CVR in comparison to the right hippocampus. We also observed a significant positive correlation between left hippocampal CVR and task performance in the Mnemonic Discrimination Task, assessed by lure discrimination index (LDI). Our results show that early changes in vascular integrity seem to differentially affect left and right hippocampus

and stress the relevance of hippocampal vascular integrity in hippocampus-related memory performance.

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Poster

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Topic: H.07. Long-Term Memory

Support: NIH Grant R01 AG070592

Title: Histological delineation of the entorhinal, perirhinal, and parahippocampal cortices for the development of a harmonized segmentation protocol for 3T MRI

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Abstract: The magnetic resonance resonance imaging (MRI) segmentation protocols for boundaries of the entorhinal, perirhinal, and parahippocampal cortices vary significantly across laboratories. Such variability leads to discrepancies in interpreting structural and functional neuroimaging results and when comparing them across studies. Moreover, the lack of a consensus protocol for areas of the brain affected by tau pathology, such as the perirhinal region, is a major barrier for applying structural MRI to early detection of Alzheimer's disease. An international working group has been formed to create a valid, reliable protocol for the segmentation of the entorhinal, perirhinal, and parahippocampal cortices that can be used for in vivo 3 tesla structural MRI. To ensure validity of the harmonized protocol, four independent

laboratories used digital histology images to annotate medial temporal lobe divisions based on cytoarchitecture. Three Nissl-stained cases were used: Case 1: 90 year old male, normal control, right hemisphere; case 2: 66 year old female, normal control, left hemisphere; case 3: 83 year old female, progressive supranuclear palsy, right hemisphere. Nissl-stained series were prepared perpendicular to the long axis of the temporal lobe, beginning at the temporal pole. Among the three cases, the collateral sulcus varied in the depth and length, thus allowing the evaluation of variability in subregions in relationship to macrostructural landmarks. Four neuroanatomists (Augustinack, Ding, Insausti, and Kedo) identified boundaries for the entorhinal cortex, perirhinal cortex, and the parahippocampal cortex on Nissl-stained sections 5 mm apart, resulting in a total of 15-16 digitized slices per case. This collaborative effort reduces the uncertainty among boundaries in published atlases, which were based on different cases and staining procedures. Boundary agreement among neuroanatomists was high in the entorhinal cortex and Brodmann Area 35, especially in the two cases with a shallow collateral sulcus. Agreement was lower in Brodmann Area 36 and the parahippocampal cortex due to differences in how specific histological criteria were weighted (e.g. cell density and layer characterization), particularly in transitional zones. These segmented histological cases will be used to guide the development of a new harmonized protocol for the MTL cortex subregions that can be applied to in vivo MRI.

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Poster

657. Human Learning and Memory

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Topic: H.07. Long-Term Memory

Support: NIH Grant R01-HL163844

Title: Hippocampal Subfields Group progress update: Consensus protocol to segment subfields within the hippocampal body on high-resolution in vivo MRI

Authors: *A. DAUGHERTY[1], S. SAIFULLAH[1], J. C. AUGUSTINACK[2], K. M. AMUNTS[3], A. BAKKER[5], D. BERRON[6], T. I. BROWN[8], A. BURGGREN[9], G. CHETELAT[10], R. DE FLORES[11], S.-L. DING[12], R. INSAUSTI[13], O. KEDO[4], R. LA JOIE[14], N. MALYKHIN[15], A. MARTINEZ[16], S. MUELLER[14], R. K. OLSEN[18], D. J. PALOMBO[19], N. RAZ[20], C. E. STARK[21], L. WANG[22], L. WISSE[7], P. YUSHKEVICH[23], V. A. CARR[17];
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Abstract: Hippocampal subfields are differentially sensitive in development, aging, and neurodegenerative disease. High-resolution imaging techniques have accelerated clinical research of hippocampal subfields; however, substantial differences in protocols impede comparisons in the literature across laboratories. The Hippocampal Subfields Group (HSG) is an international organization seeking to address this issue by developing a histologically-valid, reliable, and freely available segmentation protocol for high-resolution T2-weighted 3T MRI (http://www.hippocampalsubfields.com). This progress update presents the consensus draft protocol for segmenting subfields within the hippocampal body. The segmentation protocol is based on a novel histological reference data set labeled by multiple expert neuroanatomists. Two naïve raters demonstrated feasibility on an MRI dataset including brains from children and adults, and all subfield volume measurements had good reliability. Twenty-six labs with reported 4 years or more experience segmenting hippocampal subfields in healthy lifespan and patient populations participated in an online survey, which included detailed protocol information, feasibility testing, demonstration videos, example segmentations, and labeled histology. Due to the complexity of the internal anatomy, two approaches for segmenting the boundary between cornu ammonis (CA) 3 and dentate gyrus subfields were presented, and the majority approved a geometric heuristic-based protocol over one that referenced the endfolial pathway anatomy: 58% geometric, 23% endfolial, and with 19% expressing no opinion. Labs rated each internal boundary definition for clarity and agreement with the protocol on a scale 1 (low) to 9 (high). All definitions were rated with high clarity (M = 8.42 - 8.65) and reached consensus agreement (binomial ps < 0.01). The geometric heuristic protocol includes labels for the internal boundaries between subiculum, each CA field, and dentate gyrus, which when combined with the external boundaries that previously reached consensus, labels subfield volumes throughout the hippocampal body. We are now conducting a formal reliability test of the hippocampal body protocol with a group of expert and novice raters who are naïve to the protocol. With confirmation of reliability, we will disseminate the validated harmonized segmentation protocol and resources for automated segmentation. The harmonized protocol will significantly facilitate cross-study comparisons and provide increased insight into the structure and function of hippocampal subfields across the lifespan and in disease.

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Poster

657. Human Learning and Memory

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Program #/Poster #: 657.08

Topic: H.07. Long-Term Memory

Support: NSF1728764

Title: The role of mnemonic style and medial temporal lobe connectivity in protecting memory from misinformation

Authors: A. S. RATZAN[1], M. D. SIEGEL[1], J. M. KARANIAN[2], A. K. THOMAS[1], *E. RACE[1];
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Abstract: Individuals differ in the degree to which they rely on episodic or semantic memory when reconstructing autobiographical memories. These intrinsic biases in mnemonic style have been linked to variability in resting-state functional connectivity (rsFC) in medial temporal lobe (MTL) networks. In particular, those with an episodic bias have stronger MTL connectivity to posterior regions involved in the recovery of visual-perceptual details, whereas those with semantic bias have stronger MTL connectivity to prefrontal regions associated with controlled semantic retrieval (Sheldon et al., 2016). Here, we sought to explore whether such variability in rsFC also relates to memory accuracy in the face of misinformation. Prior research has shown that accurately recalling memories in the face of misinformation involves retrieval of episodic details associated with a target memory (e.g., perceptual details) and reactivation of cortical regions associated with that memory (e.g., visual cortex) (Karanian et al., 2020). Thus, we predicted that individuals with stronger intrinsic connectivity in MTL-visual networks associated with an episodic retrieval style would demonstrate better protection from misinformation. Participants completed a memory task in which memory for a video event was tested after exposure to misleading verbal details. Intrinsic rsFC was measured in MTL networks using the parahippocampal gyrus (PHG) as a seed. Participants also completed the Survey of Autobiographical Memory (SAM) to measure episodic versus semantic retrieval style. At the behavioral level, we found that an episodic retrieval style was associated with better memory accuracy in the face of misinformation. At the neural level, we first replicated the finding that participants with an episodic retrieval style have increased PHG connectivity to perceptual regions, such as the precuneus, while those with semantic bias have increased PHG connectivity to prefrontal regions, such as the dlPFC. Importantly, performance on the misinformation memory task was associated with rsFC in a network that overlapped with that linked to episodic retrieval style, including connectivity between PHG and visual association areas (BA 19). However, protection from misinformation was also associated with increased rsFC between PHG

and frontal regions involved in controlled memory retrieval (BA 44/9). These results suggest that stronger intrinsic connectivity in MTL-visual networks associated with episodic retrieval style may protect individuals from misinformation, but that intrinsic connectivity in broader MTL networks may also improve memory accuracy in the face of misinformation.

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Poster

657. Human Learning and Memory

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Topic: H.07. Long-Term Memory

Support: NIH-NINDS 2R01 NS089729
F31NS126016

Title: Repulsion of overlapping memory representations in the hippocampus depends on internal beliefs about the environment

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Abstract: The hippocampus is believed to play a critical role in disambiguating memories for similar events (Yassa & Stark, 2011). Consistent with this view, pattern-based fMRI studies have found that event similarity triggers an active "repulsion" of hippocampal representations (Chanales et al., 2017; Favila et al., 2016; Hulbert and Norman, 2014). Moreover, this repulsion is time-locked to successful behavioral discrimination of similar memories (Wanjia et al., 2021). One account of these findings is that repulsion of hippocampal representations specifically occurs when external stimuli are similar, but internal beliefs are distinct (Sanders et al., 2020). Here, we tested this idea using a spatial learning task in which human participants learned four different routes within the University of Oregon campus. Each route consisted of a series of sequentially presented images terminating at a specific destination (24 s, total). Critically, although each route terminated at a unique destination, the four routes contained two pairs of competing routes. For the first 6 s, the competing routes were identical (SAME segment); for the next 12 s, the routes followed identical paths but there were subtle differences in the images (OVERLAPPING segment); for the final 6 s, the routes followed divergent paths and were visually distinct (NON-OVERLAPPING segment). Importantly, to manipulate internal beliefs, each route was preceded by a probabilistic cue (75% valid) indicating the likely destination of the route. Thus, during the SAME segment, the competing routes were objectively identical, but beliefs were manipulated via the cues. Preliminary fMRI pattern similarity analyses (n = 6) revealed that during the SAME segment—when competing routes were identical—there was a cue-driven repulsion effect in the hippocampus (selective to CA2, CA3, and dentate gyrus).

Namely, when cues indicated different destinations (but the visual stimuli were identical), pattern similarity between competing routes was actually lower than pattern similarity between non-competing routes. Repulsion effects were also present during the OVERLAPPING segment (when competing routes were highly similar, but not identical); however, this effect was less dependent on the cues. Strikingly, repulsion effects were absent during the NON-OVERLAPPING segment (when competing routes were distinct). Repulsion effects were also absent, during any segment, in visual cortex. Together, our initial findings reveal that repulsion of hippocampal representations specifically occurs when visual stimuli are highly similar—or even identical—but beliefs about these stimuli are distinct.

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Poster

657. Human Learning and Memory

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Program #/Poster #: 657.10

Topic: H.07. Long-Term Memory

Support: K23NS110920
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Title: A hippocampal role in long term verbal knowledge retrieval

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Abstract: The hippocampus is known to be crucial for episodic memory, but emerging evidence suggests that, in the intact brain, it may lend a role in the retrieval of long-term factual knowledge as well. We evaluated hippocampal activity during neural processing of natural language prompts (audio of fact-based questions) eliciting retrieval target responses (single word answers) from memory stores. Participants (N=23) undergoing intracranial electrophysiology for refractory epilepsy performed an adapted auditory naming task (AAN) we tailored in order to enhance behavioral timing precision of step-wise semantic processing. For positive and negative controls of hippocampal processing, participants also performed free recall and repetition tasks, respectively. The AAN task elicited widespread cortical high gamma band activity during the course of stimuli particularly in the superior temporal, middle temporal, and inferior frontal gyri. Hippocampal activity increased toward the end of AAN sentences and prior to speech onset, and the majority of electrodes with statistically significant activity overlapped with significant electrodes in the free recall task. No hippocampal electrodes were significant in the repetition task. Lexico-semantic processing load was indexed by a new Mturk-crowdsourced metric, the number of unique answers to each AAN stimulus, which strongly predicted behavior (correct responses and reaction time) in the participants. A linear regression model showed that NUMU

explained variance of hippocampal neural activity, particularly between the end of the question stimulus and the participant's speech response. Together these results demonstrate that a relatively intact hippocampus may contribute similarly to episodic memory and long-term factual knowledge retrieval.

Disclosures: J.K. Kleen: None. **M.K. Leonard:** None. **E.F. Chang:** None.

Poster

657. Human Learning and Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 657.11

Topic: H.07. Long-Term Memory

Support: NIMH Intramural Research Program ZIA MH002920 to AM
NIMH R01 MH060941 to DS

Title: The role of the hippocampus in the construction of recent and remote autobiographical memories

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Abstract: Recent evidence suggests that the hippocampus contains functionally distinct subregions along its long axis that differentially contribute to autobiographical memory retrieval. The retrieval of autobiographical memories is thought to consist of two phases: a memory construction phase where a memory is searched for and retrieved, followed by an elaboration phase where the memory is re-experienced in detail. This dynamic retrieval process has not been investigated in the context of the hippocampal subregions as memories age. In the present fMRI study, we analyzed data from 40 participants who used picture cues to construct and overtly elaborate upon autobiographical memories from various timepoints. As a control task, participants selected a picture cue to describe. We previously reported a temporal gradient in the posterior hippocampus during the elaboration period of autobiographical retrieval, with reduced posterior hippocampal activation at remote timepoints that did not differ from the control task. The anterior hippocampus was not reliably active compared to the control task at most timepoints. Here, we examine the formerly unanalyzed, construction period of retrieval. We extracted percent signal change from native-space anterior and posterior hippocampal masks during the construction phase of each recall period relative to the control task. We used a repeated measures ANOVA to model percent signal change during memory construction as a function of condition (recall today/recall 6-18 months ago/recall 5-10 years ago), long-axis region (anterior/posterior hippocampus), and hemisphere (left/right). Unlike our previous work, during memory construction, we found no evidence of a temporal gradient. Instead, we observed

strong anterior hippocampus activity compared to the control task regardless of memory remoteness. In addition, there was a significant main effect of long-axis ($F(1,39)=7.76$, $p=0.008$), driven by greater activation in the anterior hippocampus than the posterior hippocampus. The posterior hippocampus was not reliably active at most timepoints. Our findings suggest a unique contribution of the anterior hippocampus to the construction process of autobiographical memory retrieval over time, and highlight that retrieval processes, which have yet to be fully integrated with models of systems consolidation, offer novel insights to hippocampal subregion function over time.

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Poster

657. Human Learning and Memory

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 657.12

Topic: H.07. Long-Term Memory

Title: Time is represented by global changes in entorhinal and hippocampal whole-brain functional connectivity patterns

Authors: *J. WANG[1], A. TAMBINI[2], L. PRITSCHET[1], C. M. TAYLOR[1], E. G. JACOBS[1], R. C. LAPATE[1];
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Abstract: Time elapses linearly and continuously, but our temporal memories are often fragmented and modulated by contextual shifts, such as those produced by changes in space. Multivariate neural activity patterns in the hippocampus and entorhinal cortex (EC) represent conjunctive spatial-temporal context information (Deuker et al., 2016; Bellmund et al., 2019)—however, whether they encode time independently of space remains unclear. Prior rodent studies indicate that hippocampal place cell firing patterns become increasingly *dissimilar* when the same task is performed over progressively longer temporal intervals, suggesting that gradual, task-irrelevant changes in temporal context can be dissociated from spatial context in the hippocampus (Mankin et al., 2015). In humans, changes in global states have been found to be reflected by changes in the similarity of whole-brain, multivariate functional connectivity patterns (Tambini et al., 2017). Here, we examined whether changes in temporal context can be similarly assayed by examining changes in the similarity of hippocampal and EC whole-brain multivariate functional connectivity. To do so, one participant (age=23) underwent daily acquisition of whole-brain, 10-min resting-state EPI scans ($2mm^3$ isotropic) for 30 consecutive days. T1 and T2-weighted anatomical scans were also acquired and used to derive high-resolution EC and hippocampal ROI masks, which were manually verified by a neuroanatomist. We examined whole-brain, EC and hippocampal multivariate functional connectivity across the

30-day period. As a control site, we examined changes in whole-brain multivariate functional connectivity patterns of the primary motor cortex (M1) over the same time period. We found that the similarity of multivariate, whole-brain functional connectivity patterns of both EC and hippocampus decreased linearly and significantly over time, suggesting that a slow-drifting temporal context is detectable in human resting state fMRI data. In contrast, the similarity of M1-whole-brain functional connectivity patterns did not correlate with elapsed time, and was significantly different from the time-dependent association observed when examining whole-brain functional connectivity obtained from EC and hippocampal sites. In summary, these results indicate that activity in EC and hippocampus modulates global, whole-brain processing of temporal context independently of spatial context at long time scales (lasting up to one month), and therefore may reflect the coding of time.

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Poster

657. Human Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 657.13

Topic: H.07. Long-Term Memory

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Arizona Biomedical Research Commission Grant ADHS16-162413
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Title: Baseline brain correlates of accelerated visual memory decline in middle-age and older adults with autism: the case for hippocampal free-water

Authors: M. WALSH[1], E. OFORI[2], B. A. PAGNI[3], K. CHEN[6], G. SULLIVAN[4], ***B. BRADEN**[5];
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Abstract: Introduction: Research aimed at understanding cognitive and brain aging in adults with autism spectrum disorder (ASD) is growing, but critical longitudinal work in middle-age and older adults has yet to emerge. Adults with ASD struggle with tasks involving visual memory compared with neurotypical adults (NT). This may be related to differences in size or integrity of the hippocampus and its' primary structural connectivity pathway, the fornix. The aim of this study was to describe longitudinal aging trajectories in short- and long-term visual memory abilities in middle-age and older adults with ASD, compared with matched NT adults. We also evaluated baseline multi-modal imaging metrics of the hippocampal system, including

the relatively novel metric free-water, as predictors for longitudinal memory change. **Methods:** Middle-age and older adults with ASD (n=25) and matched NT adults (n=25) between the ages of 40 and 70 years were followed longitudinally at approximately two-year intervals (range 2-5 years). Participants completed the Wechsler Memory Scale III Visual Reproduction task. Longitudinal mixed models were run to detect group differences in memory change with baseline age and sex as covariates. Hippocampal volume was measured via T1-weighted MRI images with FreeSurfer. Fornix FA and hippocampal and fornix free-water were measured from diffusion tensor imaging (DTI) scans. Correlations were run between individual hippocampal system metrics and longitudinal memory change slopes. Alpha was set at 0.05. **Results:** There was a significant group by time interaction for long-term visual memory, such that middle-age and older adults with ASD declined faster than matched NT adults (p=0.036). There was no group by time interaction for short-term visual memory. Baseline free-water in the hippocampus was the only hippocampal system metric that correlated with long-term visual memory change in middle-age and older adults with ASD (p=0.043). **Discussion:** In one of the first longitudinal cognitive and brain aging studies in middle-age and older adults with ASD, findings suggest vulnerabilities to accelerated long-term visual memory decline, compared to matched NT adults. Further, baseline free-water of the hippocampal system may be a predictor of memory change in middle-age and older adults with ASD. This relatively novel microstructure metric is thought to indicate atrophy in a way that is more sensitive than total structure volume. These preliminary findings lay the groundwork for future prognostic applications of MRI for cognitive aging in middle-age and older adults with ASD.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 658.01

Topic: H.08. Learning and Memory

Support: DARPA # HR0011-18-2-0021
NIH R01 NS12176401

Title: Rapid learning of new information using Similarity-weighted interleaved learning (SWIL)

Authors: *R. SAXENA[1], Z. NAVRATILOVA[2], J. L. SHOBE[1], B. L. MCNAUGHTON[1]; [1]Univ. of California, Irvine, Irvine, CA; [2]Univ. of California Irvine, Irvine, CA

Abstract: The artificial neural networks (ANNs) tend to abruptly lose previously acquired knowledge while the information from a new item is being incorporated all at once into the network, demonstrating catastrophic forgetting. On the other hand, our brains can continually learn, fine-tune, and transfer knowledge throughout their lifespan. How does the brain achieve

this? Complementary Learning Systems Theory suggests that new item information can be gradually integrated into the neocortex by interleaving novel information with existing knowledge. However, this approach is extremely time-consuming and data-hungry, requiring interleaving all existing knowledge every time something new is learned. In the current study, we used attractor networks and deep, nonlinear ANNs to learn new items by interleaving only a subset of old items with high similarity to the new item. We chose to retrieve the previously learned class exemplars based on their similarity with the new item, as the performance on highly similar old items will be most negatively impacted by new item learning. By using such similarity-weighted interleaved learning (SWIL), we could reach performance levels comparable to that achieved by using the entire training dataset, thereby reducing the amount of data required and learning time, making SWIL a fast and data-efficient approach. The attractor network model was based on a persistent excitability tag determined by the amount of overlap of the new item with the old class ensembles. For both modeling approaches, we also show that new item learning speed scales in proportion to the number of non-overlapping classes learned previously. These results might help build better continual learning algorithms and provide insights into possible hippocampus-cortex interaction during memory consolidation.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.02

Topic: H.08. Learning and Memory

Support: DARPA # HR0011-18-2-0021
NIH R01 NS12176401

Title: Global and rate remapping in retrosplenial cortex

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Abstract: Semantic memory and abstraction of knowledge from experience are thought to involve communication between the hippocampus and neocortex. The hippocampus encodes novel episodes using sparse and orthogonal neural representations, which form and stabilize in ~10 minutes of exploration of a novel environment. Recent studies have found that several neocortical areas, including the superficial layers of retrosplenial cortex (RSC), contain sparse neural coding correlated to spatial location in one-dimensional environments. These cortical neural representations are highly similar in sparsity and field width to those in dorsal

hippocampus (CA1), and do not form after hippocampal lesions. This suggests that the hippocampus projects spatial information out to many neocortical areas. We set out to discover if RSC pyramidal cells, like CA1 neurons, would rapidly form spatial representations in novel (virtual) environments. We found that upon entry into a novel environment, the activity of RSC neurons, like CA1 neurons, immediately became de-correlated from the activity in the familiar environment ("global remapping"). Over the course of 10-15 laps, activity became more consistent lap to lap, eventually showing spatial correlations similar to those in the familiar environment. In that study, visual objects differed between environments, but within an environment were always found in the same locations. Thus, "spatial tuning" could not be distinguished from "visual tuning." In the next experiment, we addressed this by shuffling the positions of a subset of objects on every lap. We found that many fewer cells, in both CA1 and RSC, showed consistent spatial firing fields along a section of the track in which objects were shifted on every lap. At the same time, very few cells were consistently active at any individual shifting object, therefore also showing a lack of visual control over RSC activity. Finally, we introduced the object shuffle manipulation after animals had already learned the same environment in a stable configuration ("destabilization"). Unlike in the shifting (from start) condition, RSC cells continued to show spatially correlated activity, despite the locations no longer being visually identical across laps. Previous studies have shown that following minor changes to a familiar environment, CA1 cells tend to change their firing rates (up or down), without changing the locations where they fire ("rate remapping"). We found that after destabilization, most cells continued to fire in the same locations, but increased the variability of their peak activity, over different cue configurations, as would be expected for rate remapping.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.03

Topic: H.08. Learning and Memory

Support: NIA R00 AG049090
FL DOH 20A09
R01 AG070094

Title: Rescuing impaired cortical-hippocampal interactions during sleep with 40Hz stimulation targeted to hippocampus in 3xTg-AD/PVcre mice

Authors: S. D. BENTHEM, S. MOSELEY, J. DIXON, A. SILK, A. C. STIMMELL, A. A. WILBER;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: In preclinical Alzheimer's disease (AD), spatial learning and memory is impaired (Allison et al., 2016). We reported similar impairments in 6-month 3xTg-AD female mice on a virtual *spatial reorientation task* that requires memory for using landmarks to orient in space (Benthem et al., 2020). Memory replay during sleep is critical for learning related plasticity (Ego-Stengel & Wilson, 2009; Jadhav et al, 2012; Maingret et al, 2016), and hippocampal-cortical dysfunction is a potential mechanism for memory impairments in AD (Gennaro et al, 2017; Khan et al, 2014). Consistent with this hypothesis, we previously found deficits in hippocampal-parietal cortex (HPC-PC) coordination during sleep coinciding with impairments on the virtual maze (VM) *spatial reorientation task* (Benthem et al, 2020). Gamma-stimulation (40Hz) has been shown to clear AD pathology in mice (Iaccarino et al, 2016; Martorell et al, 2019), and improve functional connectivity in preclinical AD patients (He et al, 2021). Thus, we assessed HPC-PC coordination in 3xTg-AD/PV^{cre} mice learning the same task. We implanted a 16-tetrode recording array targeting PC and HPC and an optical fiber targeting HPC. Daily recording sessions of rest-task-rest commenced as mice learned to locate the unmarked reward zone. During the same surgery, cre-dependent AAV was used to express channel rhodopsin 2 in hippocampal interneurons. This allowed for daily hippocampal stimulation sessions after each recording session, with either 40Hz or SHAM stimulation. We assessed sleep quality metrics, spindles, delta waves (DW), and pattern reactivation in PC, and hippocampal markers of memory replay (SWRs) during slow wave sleep (SWS). Animals with 40Hz entrainment showed decreased SWS sleep compared to SHAM animals. Finally, in SHAM stimulated mice SWR-DW cross-correlations were reduced, similar to 3xTg-AD mice (Benthem et al, 2020); while in the 40Hz stimulated mice, this phase locking was rescued. Furthermore, the 40Hz stimulated mice have at least partially restored performance on the VM compared to SHAM mice. However, this rescued HPC-PC coupling no longer predicted performance as in NonTg animals (Benthem et al, 2020). Instead, SWRs and DWTs independently predicted performance on the VM in 40Hz animals, but not SHAM animals. Thus, 40Hz stimulation of hippocampus may rescue learning and memory related functional interactions in the hippocampal-PC network during sleep and as a consequence partially rescue impairments in spatial navigation, despite a decoupling between HPC-PC coordination during sleep and learning and memory.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Support: F32 MH099682
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MH46823-16
Alberta Innovates Health Solutions Fellowships

NIA K99/R00 AG049090
FL DOH 20A09

Title: Freely available novel behavioral platform for assessing spatial navigation and reference frame transformations

Authors: *A. BREA GUERRERO[1], M. OIJALA[2], S. MOSELEY[3], T. TANG[3], F. FLETCHER[3], B. L. MCNAUGHTON[5], A. A. WILBER[4];
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Abstract: Spatial navigation researchers use a wide range of behavioral paradigms to assess and tax the activity ofthe neuronal navigation system. Some examples are the Morris water maze, Barnes maze, and Oasismaze. Developing flexible hardware and software that permits assessing a variety of spatial navigationstrategies and learning paradigms is critical, particularly when the objective is to isolate thecontributions of brain regions and networks to spatial behavior. We developed a platform, that we aremaking freely available, to allows users to build a variety of behavioral paradigms using up to 32 spatialgoal locations. We have used this approach to assess neural activity patterns using five behavioralparadigms and to integrate data collected with two different recording platforms (Neuralynx andOpenEphys) in mice and rats. The platform allows for automatic dispensing of a liquid reward at up to 8reward locations, or brain stimulation reward at up to 32 reward locations; delivered upon entry intouser-defined spatial locations. For video acquisition and automatic triggering of maze events by trackingthe animals' location, the system can be integrated with video acquisition features of either Cheetahincluded in Neuralynx recording systems, or Bonsai integrated with the OpenEphys platform. Theapparatus has 32 evenly spaced LEDs around the perimeter of the platform. These LEDs can serve as cuelights and also to auto-define spatial zone locations at reward locations. All spatial zone locations timestamp (including the zone ID) zone entry and exit, and all maze events (cue light onset and offset,reward onset and offset, etc) are automatically timestamped using the Neuralynx or OpenEphys clocks.An Acces I/O USB-IDO board serves as interface to control the platform's electronics *via* code written inMatlab. Here we present data collected from two previously published behavioral tasks (Bower et al.,2005; Rosenzweig et al., 2003; Wilber et al., 2017; Stimmell et al., 2019; Benthem et al., 2020), and twonew tasks that we developed to assess coordination between body-centered and world-centeredreference frames. We also present the use of this platform for recording electrophysiological data fromthe hippocampus and parietal cortex. We used silicon probes or tetrodes to collect thiselectrophysiology data, while freely moving mice and rats performed these tasks. This inexpensive andnovel approach granted us the flexibility to develop and carry out several behavioral paradigms withoutadditional program or maze construction and has the potential to be configured for many moreparadigms designed by future users.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.05

Topic: H.08. Learning and Memory

Support: NIA R00 AG049090
FL DOH 20A09
R01 AG070094
NIAAA R01 AA029700

Title: A Hippocampal-parietal Network for Map to Action Transformation

Authors: *Y. ZHENG[1], X. ZHOU[1], C. SIMMONS[1], S. MOSELEY[1], A. KLASCHUS[1], R. THÉ[1], E. JOSEPH[1], B. J. CLARK[2], W. WU[1], A. A. WILBER[1];
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Abstract: Movement through space and establishing memories based on such experiences is essential for survival of animals including humans. This ability is thought to require storage of memories often in an allocentric (map-like) framework and conversion to a body-centered reference frame comprised of specific locomotor actions (e.g., turn right). These frameworks must be coordinated in a fluid manner during navigation. The encoding observed in hippocampus (HPC) and parietal cortex (PC) has led to the notion that this circuit operates as a part of a coordinate transformation network; for example, transforming a remembered allocentric representation into the appropriate action. While HPC neurons are typically modulated by allocentric locations , PC neurons are modulated by multiple reference frames including actions (e.g., right turn). . This hypothesis was tested using a *complex spatial sequence task* where rats learn to navigate to unmarked locations fixed in space in a specific sequence. Landmarks are distributed around the room for spatial orientation. We use a sequence (**1-2-3**-4-**1-2-3**-5-) that has a repeating path segment (**1-2-3**) followed by one of two distinct actions. Specifically, the rat learns in context **1-2-3**-4, to go to 4 for reward, while in context **1-2-3**-5 the rat must go to 5. Thus, navigation to zone 4 or 5 requires a map-to-action transformation. This emulates the spatial memory problem one encounters when driving through an intersection and remembering the appropriate action given the current route and goals (e.g., turn left to a bank versus right to home). Sets of 'cued' runs in which a light at each goal leads the rat through the sequence are interleaved with unguided ('memory') runs through the complete **1-2-3**-4-**1-2-3**-5 sequence . During memory runs, following an error, a light cue directs the rat to the next zone in the sequence. Thus, during memory runs, the rat must coordinate between remembered allocentric context and egocentric action. We found that decoded PC ensembles (and some single cells; but not simultaneously recorded HPC ensembles) predict upcoming actions following the repeating path segment (**3**-4 & **3**-5) during 'memory' but not 'cued' trials. Additionally, we hypothesize that accurate action selection by PC will require HPC encoding of *memory for the allocentric context*. Consistent with this hypothesis, we found that a failure in allocentric context decoding (forgetting if came from 5 vs 4 while navigating 1-2) by HPC ensembles (but not by PC ensembles) also predict behavioral errors. Thus, we provide evidence that the HPC-PC circuit is

a coordinate transformation network for interfacing between map like and person centered reference frames.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.06

Topic: H.08. Learning and Memory

Support: NIA R00 AG049090
FL DOH 20A09
R01 AG070094

Title: Getting oriented in 'new surroundings' is impaired during early alzheimer's disease pathogenesis and these impairments are reversed by sleep

Authors: A. C. STIMMELL[1], ***J. MARQUEZ DIAZ**[2], S. D. CUSHING[3], S. MOSELEY[4], A. A. WILBER[3];
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Abstract: An essential component of productive memory consolidation and waste productclearance, including Aβ and tau associated with Alzheimer's disease (AD), is sleep (Tononi etal., 2014; Lucey 2020). Poor sleep quality, short sleep duration and disrupted slow wave sleep,are associated with increased amyloid beta (Aβ) and tau, poor cognition, and an increased risk ofAD (Nebel et al 2018). Conversely the facilitation of sleep decreases Aβ and tau accumulation(Zhao et al., 2019). Sleep is also important for the consolidation of memories, including spatialmemories. Sleep events associated with memory related brain dynamics are disrupted in 3xTg-AD female mice and contribute to impaired spatial navigation behavior (Benthem et al 2020).Thus, improving sleep may offer a multifaceted approach to improving cognition and slow orhalt disease progression in those at risk for AD; however, studies assessing sleep impacts on ADoften fail to assess cognition.Getting lost, particularly in new surroundings, is an early cognitive impairment inhumans that will develop AD (Allison et al., 2016). Recent work including our own suggests thatthis early impairment, getting lost in new surroundings, could represent a failure to use distalcues to reorient in space (Stimmell et al., 2019). Thus, we set out to assess the impact of sleep onimpaired spatial reorientation that we previously observed in 6-month female 3xTg-AD mice.We randomly assigned 3xTg-AD mice to a sleep group (n = 7; 50 min pre- and post-task sleepsessions) or a non-sleep group (n= 7; mice remained in the colony in their home cage before andafter the task). Mice in both groups were compared to non-Tg age matched controls that alsospent the pre- and post- task period in their home cage (n=7). Finally,

to confirm that our sleepcondition induced sleep we performed the same experiment in 3xTg-AD and control mice(n=5/group) implanted with a 16-tetrode recording array targeting the hippocampus and parietalcortex for recording local field potentials to classify sleep states. This additional experiment alsoallowed us to assess markers of memory consolidation (delta waves, sharp wave ripples, corticalspindles). Markers of pathology were assessed in all mice (pTau, 6e10, M78, M22, as in Stimellet al., 2020). We found that 6-month female 3xTg-AD sleep mice (both with and without arecording array) were not impaired at spatial reorientation compared to 6-month female non-Tgcontrol mice. While 6-month female 3xTg-AD no sleep mice were impaired at spatialreorientation learning, a replication of Stimmell et al., 2019. Thus, improving sleep in earlystages of AD pathology offers a promising approach for improving cognition.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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FWF J 4271 to AC

Title: Encoding Temporal Traces within Hippocampal-Cortical Circuits

Authors: *A. CICVARIC[1], T. BASSETT[1], Z. PETROVIC[1], N. YAMAWAKI[2], L. Y. REN[3], V. JOVASEVIC[3], A. L. GUEDEA[3], Y. HAN[4], V. GRAYSON[3], G. M. SHEPHERD[3], J. M. RADULOVIC[1,3,2];
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Abstract: Episodic memories are integrated autobiographical records of events, both of their temporal relationships, as well as places where they occurred. Here, we employed trace fear conditioning paradigm (TFC), in which an animal learns to associate two stimuli that are separated by temporal trace, in combination with chemogenetic and fiberphotometry approaches to study mechanisms encoding the temporal trace. It has been widely accepted that excitatory neurotransmission in the dorsal hippocampus (DH) and several cortical areas, including retrosplenial cortex (RSC) play a key role in the formation of spatial and temporal episodic-like memories. In our previous work we showed that DH→RSC projections can be split into two molecularly distinct populations; projections that are expressing exclusively VGLUT1 or VGLUT2. Here we showed that both types of projections redundantly contributed to the formation of tone-trace-shock representation, but only VGLUT2-containing terminals were

necessary for the processing of the trace itself. Additionally, we showed that post-tone freezing deficits in TFC, when VGLUT2 containing projections were silenced were not due to the impaired processing of sequences of stimuli but were likely specific to the processing of the temporal trace. Furthermore, inhibition of GAD-2 positive RSC interneurons, resulted in impaired freezing during the trace. This indicates postsynaptic involvement of interneuronal population of the RSC in the trace processing. We are currently investigating, using fiber photometry, whether differences in the activity of these projections and associated activity of RSC neurons contribute to their differential involvement in TFC.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 658.08

Topic: H.08. Learning and Memory

Support: NIMH MH108837
NIMH MH078064

Title: Developmental change of hippocampal-dependent contextual fear memory

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Abstract: The persistence of hippocampal-dependent memory requires the interaction between hippocampus and cortex. During the postnatal developmental period, the synaptic connections between brain regions are formed and stabilized. The aim of this ongoing study is to investigate the developmental change of hippocampal-dependent contextual fear memory and its relationship with dorsal hipppcampus (DH) - retrosplenial cortex (RSC) circuit development. Our results showed that as early as p28-p32, mice were able to form specific contextual fear memory. The retrieval of contextual fear memory formed on p29 decreased throughout development from p30 to p61. Chemogenetic reactivation of the neurons active during p29 fear conditioning in RSC partially recovered those memories in adulthood. We further found that the expression of perineuronal nets (PNNs) in the DH and RSC changed with age and that TGF-beta signaling is associated with the regulation of PNN and memory retrieval. These results implied that the immature PNNs are responsible for the limited memory persistence in young mice. Future experiments will aim at the role of PNNs and TGF-beta signaling in the development of DH-RSC circuit and memory persistence.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 658.09

Topic: H.08. Learning and Memory

Title: The Effect of Cognitive Task Switching on Neural Representations in Human Frontal and Temporal Lobes

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Abstract: The process of switching between tasks is cognitively taxing, and has long been known to give rise to a "switching cost", leading to a degradation in RT and accuracy after the task switch. The neurophysiological basis of this cost is the subject of significant controversy, with multiple factors thought to play a role including interference of the previous task set and reconfiguration of frontal networks to encode the current task set, known as task set inertia (TSI) and task set reconfiguration (TSR) respectively. Significant research efforts have been dedicated to conducting behavioral and non-invasive neurophysiological studies of the effects of task switching to provide evidence for adjudication between these competing theories, and to generally shed light on the generative mechanisms for switching costs. However, to this day, almost no studies have been conducted at the single-neuron level in humans studying the neural correlates of task switching and switching costs. we conducted single-unit recordings in human patients implanted for clinical purposes who were instructed to alternate many times between two tasks, one involving a sensory-perceptual decision (Category Task) and the other involving a memory-dependent decision (Memory Task). We recorded 612 neurons in 8 patients from frontal and prefrontal cortical structures including the dorsal Anterior Cingulate Cortex (dACC), pre-Supplementary Motor Area (preSMA), and ventromedial prefrontal cortex (vmPFC), and from medial temporal lobe structures, namely the hippocampus (HPC) and the amygdala (AMY). During the task, we find that task variable representations that are un-cued and internally maintained by the individual completing the task are particularly sensitive to task-switching, being perturbed during the immediate post-switch period, unlike representations depending on sensory stimuli or motor decisions and active engagement during the task. We also find neurons that are associated with prolonged reaction time and task errors immediately following a task switch. In summary, neurons in both frontal and temporal lobes are significantly perturbed by switching tasks, and exhibit various dynamics both during baseline and stimulus processing associated with the behavioral cost of switching tasks.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

Location: SDCC Halls B-H

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Program #/Poster #: 658.10

Topic: H.08. Learning and Memory

Support: AFOSR(FA9550-21-1-0088)
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Dana Foundation

Title: A neuronal saliency map in the human amygdala and hippocampus

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Abstract: Selective visual attention is one of the most fundamental cognitive functions in humans. The major goal of selective visual attention is to direct our gaze rapidly towards objects of interest in our visual environment. Literature suggests that selective visual attention is driven by saliency of features at the pixel, object and semantic levels (Xu et. al 2014). However, not known is whether the brain encodes visual saliency across all these levels. To address this question, we recorded from 1053 neurons in the human amygdala and hippocampus from 13 neurosurgical patients. Patients freely viewed 700 complex natural scene images (3 s for each image) with more than 5000 regions annotated and multiple layers of saliency information delineated. We quantified patients' eye movements using a novel and sophisticated computational model that can comprehensively characterize stimulus-driven attention. By integrating neuronal firing rate into this model, we constructed a "*neuronal saliency map*", which reflects the tuning of a single neuron or a population of neurons when multiple saliency factors are considered simultaneously. We then visualized the distribution of saliency weights across neurons, which showed the tuning preference of neurons. We found that faces were the most preferred stimuli (42.55% neurons preferred faces), although there existed neurons tuned to each semantic category as well as low-level visual features. Notably, the neuronal saliency map was able to predict the location of the next fixation. Together, for the first time, we constructed a neuronal saliency map in the human amygdala and hippocampus using natural scene images, which comprehensively describes the tuning of amygdala and hippocampal neurons. Thus, these regions may be involved in visual saliency computations that are known to guide our visual exploration in complex environments.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.11

Topic: H.08. Learning and Memory

Support: NIH Grant U01NS117839

Title: Eye tracking and single-neuron responses associated with recognition memory task performance in humans with epilepsy

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Abstract: The ability to encode and retrieve information about events and facts enables us to use previous experience to guide behavior. Disorders of memory are common, yet treatments for memory related disorders are lacking as a mechanistic understanding of human memory is only beginning to emerge. An obstacle to determining these mechanisms is the difficulty of measuring the activity of neurons in brain areas associated with memory in humans. Here we propose to advance the understanding of episodic memory by utilizing a rare opportunity to record eye movements and activity from single neurons from the medial temporal lobe in awake behaving humans during the encoding and retrieval process. Patients with medically refractory epilepsy (N=9, M_{age} = 41.5 ±8, 77% female) admitted for intracranial monitoring of seizure activity via stereotactically implanted depth EEG electrodes (SEEG electrodes) completed the study during their inpatient stay in the Epilepsy Monitoring Unit (EMU) at University of Colorado Hospital (UCH). Patients were implanted with 1-2 Ad-Tech Behnke Fried Macro-Microwire electrode(s) in either the hippocampus or amygdala to record local field potentials (LFPs) and single neuron activity. Eye movements were recorded binocularly from a desktop mounted EyeLink 1000 Plus System (SR Research, Canada) while patients completed up to three variants of a computerized visual recognition memory paradigm. Behavioral performance will be correlated with changes in pupil diameter and gaze position, as measured by saccades, as well as with electrophysiological parameters, to derive the role of brain networks and single neurons in performing cognitive tasks related to learning and memory. Preliminary results indicate that 1) lower fixed gaze position, as measured by saccades, during encoding is associated with lower accuracy and less confidence during the recognition portion of the task, and 2) correct responses during the recognition portion of the task (i.e., correctly identifying if stimuli have been seen before) with higher confidence ratings are positively associated with a greater fixed gaze position and an increased firing rate of memory selective cells in the medial temporal lobe (MTL), supporting the notion that neurons in

the MTL are sensitive to gaze position and respond maximally only when the stimulus is directly fixated. Using cognitive and computational models to relate both behavioral performance and eye tracking processes to the underlying neural mechanisms will ultimately inform the design of targeted interventions for causal disruptions in these systems.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.12

Topic: H.08. Learning and Memory

Support: R01MH110831
U01NS117839

Title: Evidence accumulation by single units in the human Medial Temporal Lobe (MTL) during memory-based decisions.

Authors: *M. YEBRA[1], A. G. P. SCHJETAN[2], L. N. GOVINDARAJAN[3], C. P. MOSHER[1], Y. SALIMPOUR[4], T. A. VALIANTE[2], S. KALIA[2], W. ANDERSON[4], A. MAMELAK[1], U. RUTISHAUSER[1];
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Abstract: The way we make choices between different options is thought to be by evidence accumulation (EA). Such EA can be modelled as a Drift Diffusion Model (DDM). Extensive research has investigated sensory decisions. However, how we integrate mnemonic information of declarative memories to make memory-based decisions (MBD) remains unknown. We hypothesize that neurons selective for memory content in the Medial Temporal Lobe (MTL) areas in humans integrate memory-derived evidence (MDE). We test the hypothesis that EA underlies MBD. We conducted behavioral experiments and modelling while recording from single neurons to test this hypothesis. First, we conducted 3 pilot studies with 127 subjects. They were presented with faces masked with 2-D Gaussian bubble filters, which moved to cover the face to create trials with different levels of difficulty. They indicated if a shown face was Old (O), previously seen, or New (N), never seen. RT, accuracy, and confidence scaled with difficulty. We recorded 2077 single neurons across 40 sessions in 30 epileptic patients using depth electrodes in the MTL. 215 neurons were Memory Selective (MS), exhibiting significantly different responses in O vs N stimuli. We focused our analyses on 53/115 MS cells that increased their response for O relative to N stimuli that had a firing rate greater than 0.5 Hz. The firing rates of MS cells during O trials could be predicted by a DDM model fitted to the behavioral data of a given subject. We compared 3 DDMs using Hierarchical Bayesian Model

Parameter Estimation Likelihood Approximation Networks. In the 1st DDM, we fit drift rate (v), decision threshold (a), starting point (z), and non-decision time (t) parameters. In the 2nd, we let v vary by condition (new/old, easy/hard). In the 3rd both v and t varied by condition. The last outperformed the other models for both the patients and control subjects. We found that the slopes of the firing rate increased during old trials as did the non-decision time (the time elapsed until the firing rate started changing relative to baseline). These factors were predicted, respectively, by v and t as indicated by significant correlations between the model parameters and firing rate properties (r_v=.2, p_v = .03; r_t=.4, p_t =1.2 10^{-5}). Moreover, a GLM using v and t as fixed effects and session and neuron IDs as random effects significantly predicted MTL firing rate's slopes and non-decision time (p_v= .002; p_t = 1.3 10^{-6}). Together, these findings suggest that MTL MS neurons adjust their MDE integration starting time and the speed of modulation as predicted by a DDM model fit to behavior. This suggests that the N/O bubbles task shown here is well suited to the investigation of the EA process for MBD.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.13

Topic: H.08. Learning and Memory

Support: NIH Grant U01NS117839
Leopoldina Postdoc fellowship LPDS 2019-11

Title: A single cell correlate of theta-gamma phase amplitude coupling during working memory in the human hippocampus

Authors: *J. DAUME[1], Y. SALIMPOUR[2], A. G. P. SCHJETAN[3], W. ANDERSEN[2], T. A. VALIANTE[3], A. N. MAMELAK[1], U. RUTISHAUSER[1];
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Abstract: Theta-gamma phase amplitude coupling (PAC) reflects the coordination of neural activity across different frequency bands. Especially in working memory (WM) theta-gamma PAC has been observed in various brain regions, presumably reflecting interactions between cognitive control and stimulus processing activity. To date, however, there is no established single-cell correlate between local field potential (LFP)-level PAC and spiking activity of single cells. It thus remains unclear whether such "PAC neurons" exist and what role they play during WM maintenance. Here, we recorded single cell activity and population level LFPs from the human medial temporal lobe and medial frontal cortex while patients performed a Sternberg

working memory task with pictures from five different categories (44 sessions in 36 patients, 1518 neurons). We observed local theta-gamma PAC in the amygdala and hippocampus during the working memory delay period. Only in the hippocampus, PAC differed as a function of working memory load with stronger PAC observed in load 1 as compared to load 3, reflected by longer bouts of gamma activity coupled to the underlying theta rhythm with higher memory load. Weak to no PAC coupling was observed in the frontal lobe. We identified neurons in the hippocampus whose firing rate specifically followed the local interactions of theta phase and gamma amplitude during WM delay period. A significant proportion of these PAC cells were also classified as category neurons, whose firing rate was indicative of stimulus identity and showed stronger spike-field coherence to gamma when a picture from their preferred category was held in WM. PAC cells that were not also category neurons showed stronger phase coupling to remote theta oscillations recorded in ventromedial prefrontal cortex as well as stronger firing rate correlations with category neurons in load 3 than in load 1, indicating their involvement in frontal cognitive control processes that coordinate posterior stimulus maintenance during higher memory loads. Taken together, we identified neurons in the human hippocampus whose activity is related to interactions of theta phase and high gamma oscillation power. We posit that these neurons are crucially involved in both the maintenance and cognitive control processes during WM. Our results provide in-depth insights into the single cell correlates of ongoing interactions between frontal cognitive control as well as posterior sensory processing that unveil how information is maintained in WM for a brief period of time.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.14

Topic: H.08. Learning and Memory

Support: NINDS intramural research program (ZIA NS003168)

Title: Closed-loop sinusoidal stimulation of ventral hippocampal terminals in prefrontal cortex preferentially entrains circuit activity at distinct frequencies and delays

Authors: *M. MYROSHNYCHENKO[1], A. K. NTAMATUNGIRO[1], A. A. DUIN[1], D. A. KUPFERSCHMIDT[1], J. A. GORDON[2];
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Abstract: Dynamic changes in oscillatory synchrony of ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) correlate with various cognitive functions. Optogenetic inhibition of discrete neuronal projections from vHPC to mPFC has been shown in mice to

disrupt vHPC-mPFC synchrony, and optogenetic sinusoidal stimulation of these projections can facilitate vHPC-mPFC synchrony. We developed and applied open- and closed-loop optogenetic manipulations of oscillatory activity in vHPC-mPFC projections to (1) systematically characterize properties of vHPC-PFC circuit dynamics across a broad range of stimulation frequencies, and (2) explore novel approaches to enhancing endogenous vHPC-mPFC oscillatory synchrony. Adult male and female C57BL/6J mice were injected with viruses encoding either ChR2 (n = 8) or GFP (n = 5) in bilateral vHPC. At least four weeks post-injection, mice were implanted with optical fibers in bilateral mPFC, local field potential (LFP) wires in mPFC and vHPC, and stereotrodes in unilateral mPFC. LFP and single-unit activity was recorded during sinusoidal optogenetic stimulation of vHPC inputs to mPFC. Open-loop sinusoidal stimulation at frequency ranges above 8 Hz and between 20-40 Hz maximally enhanced LFP power in mPFC and vHPC, respectively. Unexpectedly, open-loop stimulation elicited maximal vHPC-mPFC coherence above 15 Hz, beyond the classical theta frequency band (~4-12 Hz). In efforts to enhance endogenous vHPC-mPFC theta synchrony, we developed a closed-loop sinusoidal stimulation approach whereby vHPC inputs to mPFC were stimulated in a manner informed by ongoing vHPC theta oscillations. In this paradigm, blue laser output to mPFC was governed by the theta-filtered (7±3 Hz), half-wave rectified vHPC LFP. Closed-loop stimulation enhanced vHPC-mPFC theta coherence in ChR2- but not GFP-expressing mice. Further, we found that delaying laser output relative to ongoing vHPC theta oscillations up to 1.5 theta cycles resulted in diminished enhancement of vHPC-mPFC coherence. In contrast, closed-loop stimulation enhanced mPFC theta power regardless of stimulation delay. These results stand to inform computational models of communication between vHPC and mPFC, and guide the use of continuously varying, closed-loop stimulation to assess how enhancing endogenous long-range neuronal communication can influence behavioral measures of cognitive function.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Topic: H.08. Learning and Memory

Support: NINDS intramural research program (ZIA NS003168)

Title: Divergent forms of in vivo plasticity between ventral hippocampal inputs and medial prefrontal cortex microcircuits in wildtype and schizophrenia-relevant model mice

Authors: *T. T. CLARITY[1], R. M. MIKOFSKY[1], M. V. MYROSHNYCHENKO[1], M. BOWEN-KAUTH[1], M. HSIANG[2,1], S. E. SILVERSTEIN[1], J. A. GORDON[1,3], D. A. KUPFERSCHMIDT[1];
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Abstract: Functional connectivity between rodent ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) supports various cognitive functions and is disrupted in schizophrenia-relevant models, including the *Df(16)A*$^{+/-}$ mouse model of the human 22q11.2 microdeletion. Inhibition of either vHPC inputs to mPFC or select mPFC interneuron (IN) populations in wildtype (WT) mice induces vHPC-mPFC dysconnectivity and cognitive deficits that mimic phenotypes observed in *Df(16)A*$^{+/-}$ mice. This phenotypic convergence raises three questions: (1) How do vHPC inputs to mPFC interact with downstream mPFC inhibitory microcircuits *in vivo*? (2) Are these interactions disrupted in disease-relevant models such as *Df(16)A*$^{+/-}$ mice? And (3) are these interactions plastic, offering a potential path to correcting circuit dysconnectivity? To address these questions, we characterized *in vivo* activity dynamics and activity-induced plasticity of discrete mPFC IN population responses to vHPC input stimulation in WT and *Df(16)A*$^{+/-}$ mice. We expressed ChrimsonR in vHPC neurons and GCaMP6f in mPFC somatostatin (SST)+, vasoactive intestinal polypeptide (VIP)+, parvalbumin (PV)+ or CaMKII+ pyramidal neurons of adult WT and *Df(16)A*$^{+/-}$ mice (n=5-13). We delivered red light pulses to mPFC to excite vHPC terminals and monitored postsynaptic GCaMP6f Ca^{2+} responses using fiber photometry. Over 50 days, mice received six "input-output" sessions of vHPC terminal stimulation at various durations/frequencies to characterize response curves for each mPFC neuron population. For 12 days early in the 50-day timeline, a subset of mice received additional high-frequency vHPC input stimulation (HFS) intended to induce plasticity of these response curves. SST+ IN responses to input-output vHPC terminal stimulation were weak at baseline in WT and *Df(16)A*$^{+/-}$ mice, but progressively increased over 50 days ($p<0.001$). This potentiation was blunted in *Df(16)A*$^{+/-}$ relative to WT mice ($p<0.001$). Repeated HFS induced some further enhancement of evoked SST+ responses. In contrast, HFS rapidly suppressed VIP+ responses to vHPC input stimulation in WT and *Df(16)A*$^{+/-}$ mice ($p<0.001$). PV+ IN responses to HFS also rapidly diminished in WT and *Df(16)A*$^{+/-}$ mice ($p<0.001$), whereas pyramidal neuron responses to HFS in WT mice were small and stable over weeks. Ongoing work is characterizing the synaptic mechanisms and behavioral effects of these divergent forms of plasticity. Together, these findings reveal properties of cell-type-specific functional connectivity and plasticity within intact vHPC-mPFC circuits that may be leveraged to influence cognition-relevant circuit function and dysfunction.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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NIH R01 5R01MH096274

Title: Sex-biased and isoform-specific rescue of working memory deficits by developmental GSK3 alpha and beta inhibition in a mouse model of schizophrenia predisposition

Authors: C. M. ALOIMONOS[1], J. PASSECKER[1,2,3], C.-Y. CHANG[3], A. DAGUNTS[1], *D. A. KUPFERSCHMIDT[1], J. A. GOGOS[3], J. A. GORDON[1,4];
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Abstract: Overactivation of the Glycogen Synthase Kinase 3 (GSK3) pathway is implicated in schizophrenia and other psychiatric disorders. Non-selective GSK3 (α and β isoform) inhibitors have been shown to rescue cognitive deficits in schizophrenia-relevant models. While such effects are often attributed to altered GSK3β signaling, relative contributions of the two isoforms are unclear. Recently developed selective inhibitors now allow targeted inhibition of each isoform. Here, we tested their potential to rescue cognitive and neurophysiological deficits in the Df(16)$A^{+/-}$ mouse model of the schizophrenia-predisposing 22q11.2 deletion syndrome. During early postnatal development (P7-P28), wildtype and Df(16)$A^{+/-}$ male and female mice were administered either the selective GSK3β inhibitor BRD3731 (n = 121) or the selective GSK3α inhibitor BRD0705 (n = 109). As adults, GSK3β inhibitor-treated mice were implanted with electrodes in ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC). Both GSK3β and α inhibitor-treated mice were trained and tested on a T-maze delayed non-match-to-sample task of spatial working memory (SWM); GSK3α inhibitor-treated mice were also tested for avoidance behavior on the elevated plus maze (EPM). GSK3β inhibition from P7-P28 rescued deficits in SWM task acquisition in adult male but not female Df(16)$A^{+/-}$ mice (3-way ANOVA, $p<0.01$). Indeed, wildtype female mice treated with the GSK3β inhibitor treatment showed impaired SWM task acquisition. *In vivo* electrophysiological recordings revealed that GSK3β inhibition also modulated SWM task-related vHPC-mPFC theta-frequency (4-12 Hz) coherence in a genotype-specific manner. Selective developmental GSK3α inhibition did not to rescue SWM task acquisition deficits, but did reverse deficits in SWM task performance under conditions of increased SWM load in male and female Df(16)$A^{+/-}$ mice. During EPM testing, Df(16)A+/- mice showed reduced open arm avoidance, and postnatal GSK3α inhibition increased open arm avoidance. Ongoing transcriptomic analysis of vHPC and mPFC tissue from wildtype and Df(16)$A^{+/-}$ mice at P7, P28 and P90 has revealed sex-, genotype- and age-specific transcriptomic profiles that will inform the molecular basis of our behavioral and neurophysiological findings. Together, our work indicates differential roles of GSK3α and β isoforms in the development of neural circuitry supporting spatial working memory and its disease-relevant dysfunction in mice.

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Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.17

Topic: H.08. Learning and Memory

Support: National Institute of Neurological Disorders and Stroke Intramural Research Program (ZIA NS003186)

Title: Keep me(dial PFC) in the loop: effects of closed-loop optogenetic stimulation on mouse hippocampal-prefrontal communication and spatial working memory function

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Abstract: Spatial working memory (SWM) is the ability to remember behaviorally relevant environmental cues over short timescales. Optogenetic inhibition studies in mice show that activity of direct vHPC projections to mPFC supports SWM and theta-frequency (~4-10 Hz) synchrony, a proposed oscillatory mechanism for vHPC-mPFC communication. While vHPC-mPFC theta synchrony is correlated with SWM performance, its causal contributions remain unclear. To explore such contributions, we developed a novel closed-loop optogenetic stimulation paradigm aimed at manipulating endogenous vHPC-mPFC oscillatory synchrony. Adult C57BL/6J male and female mice (n = 13) were injected with viruses encoding either ChR2 (n = 8) or GFP (n = 5) in bilateral vHPC. At least four weeks post-injection, mice were implanted with local field potential (LFP) wires in mPFC and vHPC, and optical fibers (with unilateral stereotrodes) in bilateral mPFC. Blue light administered to vHPC inputs within mPFC was governed by the theta-filtered (7±3 Hz), half-wave rectified vHPC LFP. This laser waveform was delivered at various phase delays relative to ongoing vHPC theta oscillations. We found that closed-loop optogenetic stimulation delivered near-synchronously ("in-phase") with vHPC theta dynamics enhanced vHPC-mPFC theta coherence in ChR2- but not GFP-expressing mice; synchrony enhancement diminished with increasing phase delays (up to 1.5 theta cycles). In separate C57BL/6J mice (n = 8 ChR2, 11 GFP), we tested the effects of our closed-loop approach on SWM, hypothesizing that in-phase closed-loop stimulation will enhance SWM performance, whereas phase-shifted stimulation will impair it. We trained mice on a delayed non-match-to-sample T-maze task using trials with different delay lengths (10, 60 sec) to vary working memory load. Once trained, mice received closed-loop stimulation on every other trial; half of stimulation trials were in-phase and half were phase-shifted by 1.5 theta cycles. Analysis of the effects of phase-shifted closed-loop stimulation on SWM performance is currently underway. Ongoing work is assessing the electrophysiological effects of closed-loop stimulation in behaving mice. Together, our preliminary results highlight the promise of closed-loop optogenetics to probe causal contributions of long-range oscillatory synchrony to cognition and behavior.

Disclosures: A.K. Ntamatungiro: None. **M.V. Myroshnychenko:** None. **A. Duin:** None. **D.A. Kupferschmidt:** None. **J.A. Gordon:** None.

Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 658.18

Topic: H.08. Learning and Memory

Support: R01 NS127128-01
Whitehall Foundation
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NSERC

Title: Hippocampal theta and gamma- band decoupling during mobility, visual search, and sleep in macaques

Authors: S. ABBASPOOR[1], A. T. HUSSIN[3], *K. L. HOFFMAN[2];
[2]Psychology, [1]Vanderbilt Univ., Nashville, TN; [3]York Univ., Toronto, ON, Canada

Abstract: Nested hippocampal oscillations in the rodent give rise to temporal coding that may underlie learning, memory, and decision making. Theta/gamma coupling in rodent CA1 occurs during exploration and sharp-wave ripples during quiescence. Whether these oscillatory regimes extend to primates is less clear. We therefore sought to identify how oscillations and their comodulations in the macaque hippocampus vary across behavioral state. To address this, we measured local field potentials and single unit activity in the hippocampus of female macaques during active visuospatial search and quiescence (N=2) and in freely-behaving macaques during alert active behavioral states and sleep (N=2). We found that, in contrast to the rodent, theta and gamma frequency bands in macaque CA1 were segregated by behavioral states. Beta2/gamma (15-70Hz) had greater power and prevalence during visual search whereas theta (7-10 Hz) dominated during quiescence (Wilcoxon rank sum test with FDR correction for multiple comparisons, $F < 0.05$). Cross-frequency interactions revealed that delta/theta band amplitude (3-8 Hz) was negatively correlated with slow gamma band amplitude (20-35 Hz; $p < 0.05$, corrected for multiple comparisons) but positively correlated with higher frequency oscillations (gamma and high-frequency oscillations, 60-150 Hz; $p < 0.05$, corrected for multiple comparisons). Bicoherence analysis also showed no significant coupling between theta and slow gamma band. We confirmed these patterns of results in two freely-behaving animals during alert active behavioral states and sleep, indicating that immobility did not account for cross-species differences. Spike phase-locking was common in three frequencies: 3-10 Hz, 20-30 Hz and 60-150 Hz bands (n = 404 isolated units, Rayleigh test $p<0.05$ and shuffling test); however, sharp-wave-ripples (SWR) contributed to most of the low-frequency phase locking (3-12 Hz), and some of the high-frequencies and>100 Hz. (SWR, N = 185,). Furthermore, using spike autocorrelograms to detect intrinsic oscillations (n = 240 cells with spike counts > 100), we found no clear examples of delta/theta oscillatory modulation resembling those described in the rat hippocampus, suggesting no systematic, detectable intrinsic oscillatory modulation in the

theta band. These results support a role for the beta2/slow gamma modulation in CA1 during active exploration, which is decoupled from theta oscillations that are prevalent in NREM sleep in primates. These findings diverge from the rodent oscillatory canon and call for a shift in focus and frequency when considering the primate hippocampus.

Disclosures: S. Abbaspoor: None. **A.T. Hussin:** None. **K.L. Hoffman:** None.

Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.19

Topic: H.08. Learning and Memory

Support: R01 NS127128-01
Vanderbilt Brain Institute
Whitehall Foundation

Title: Concept formation through contingent object categorization in macaques

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[1]Psychology, [2]Psychology, Biomed. Engin., Vanderbilt Univ., Nashville, TN

Abstract: To respond adaptively in the face of complex and changing environments, we first simplify and then we rebuild. Simplification includes generalizing across variances to form categories. These are built into concepts by identifying the regularities and contingencies across instances. Whereas foveal primates share a predilection for forming visual object categories, it's less clear that non-human primates form a similar basis for concepts, here, generalizing and forming hierarchical contingencies around classes of objects. We therefore sought to evaluate whether macaques could learn to flexibly categorize classes of visual objects as a function of the spatial context in which they are encountered. We ran three adult female macaques on each of two distinct sets of synthetically-generated, yet naturalistic visual object classes ('FauXna'). We recorded touch selection and gaze while they completed a task in which stimuli were divided into four unique object categories ('families') within a three-dimensional, continuous feature space. Only one of these families was designated the target per screen side. In a trial, arrays comprising one target-category exemplar along with one exemplar from each of the three distractor categories were presented to either the left or right side of the screen. Category prototypes were never presented. Across an average of 5.2 daily sessions (range 3-9) and 777.3 trials (range 500-1908) per set, all animals exceeded both the 25% chance rate as well as the more stringent 50% criterion ($X^2 = 847.0515$, $p<0.001$). Furthermore, the monkeys showed out-of distribution generalization by running the task on new exemplars generated by extrapolating to unexplored sections of the feature space surrounding the categories. From the very first presentation of the new stimuli, performance exceeded chance in all 6 sets and exceeded the 50% criterion in 4/6 sets. Using additional novel target stimuli in invariantly rewarded trials, monkeys preferred

stimuli most similar to the never-shown prototypes of each target category over stimuli shifted towards previously-learned exemplars. Our findings indicate that macaques can learn categories of naturalistic visual objects, flexibly assign value based on context, and generalize this knowledge to novel stimuli in a manner that suggests a preference for the centroids of these category feature spaces, despite having never been shown the prototypes. These findings set the stage for future analysis into the neural circuitry underlying concept learning in the macaque.

Disclosures: S.S. Cooper: None. **K.L. Hoffman:** None.

Poster

658. Learning and Memory: Hippocampal: Cortical Interactions III

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Program #/Poster #: 658.20

Topic: H.08. Learning and Memory

Support: R01 NS127128-01
Whitehall Foundation

Title: Embodied and embedded sequence learning in freely-moving macaques

Authors: K. F. RAHMAN[1], S. ABBASPOOR[2], K. L. HOFFMAN[2];
[2]Psychology, [1]Vanderbilt Univ., Nashville, TN

Abstract: Natural behavior needs movement. Effective movement depends on interactions between the brain, body, and environment, therefore any description of neural mechanisms of behavior absent these couplings may be limited or even inaccurate. Standard neuroscience experimental paradigms for monkeys rely on limited behavioral measures, either restricting or eliminating movement, or through the use of limited operational measures from richer behavior repertoires. Here, we sought to measure learning in macaques by allowing more natural, species-specialized exploratory movements, and by quantifying a greater range of the behavioral repertoires they generated. We tested two adult female macaques in the Treehouse (TH), an apparatus designed to track with multiple modalities the behaviors of macaques performing species-specialized cognitive tasks. The Treehouse is a 2-tiered, 5'x5'x7 enclosure equipped with 4 touchscreen 'stations' in each of two opposite corners (8 screens, total). A trial requires sequential selection of each of 4 different objects, one per touchscreen in the designated corner. Each screen's designated correct object is a distractor on the other screens, thus the monkeys must learn which screen location is the correct one. The behavior of the primates is tracked using i. synchronized measurements of touch registration, ii. DeepLabCut (DLC) markerless pose tracking from the videos, and iii. accelerometer inertial measurement units (IMU) of head yaw/pitch/roll from the wireless recording system (Freelynx) that also records broadband neural data from the hippocampus and retrosplenial cortex. Both monkeys demonstrated learning of novel 4-item sequences in under 200 trials and sometimes in under 30 trials. After retention delays of 2-4 weeks, performance was greater for the remotely-learned sequences than for novel

sequences, indicating savings due to long-term retention. Learning rate was fastest for the final item in the sequence and slowest for the first, indicating multiple learning strategies may be employed. The DeepLabCut tracking revealed changes in pose and movement economy with learning. Together with the accelerometer data, they revealed head "checking" behavior that is suggestive of vicarious trial and error learning. These multimodal metrics of free behavior in macaques, together with wireless neural recordings, are enabling a better understanding of the neural mechanisms underlying embedded, and embodied natural learning.

Disclosures: K.F. Rahman: None. **S. Abbaspoor:** None. **K.L. Hoffman:** None.

Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 659.01

Topic: H.08. Learning and Memory

Support: 5R01NS106056-04

Title: DNA methylation at retrotransposons regulates neuron eligibility to a coding ensemble

Authors: *N. MOLLÉ[1], F. TAKI[1], A. NABILA[1], M. TOTH[2];
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Abstract: While it is well established that sensory experiences are encoded in small populations of neurons in the hippocampus, the molecular mechanism that underlies ensemble coding has not yet been identified. Dynamic epigenetic modifications such as DNA methylation establish heterogeneity among genetically identical cells, and may dictate an individual neuron's eligibility to be included into a coding ensemble. Here we report that the 1-3% of dentate gyrus neurons recruited to encode an experience differ in the DNA methylation of integrated retrotransposons, as compared to the non-recruited neurons. Following a 15-minute exposure to a novel environment, we separated activated/coding (FOS+) and non-coding (FOS-) dentate gyrus granule cells from mice and profiled DNA methylation by single nucleus RRBS (Reduced Representation Bisulfite Sequencing). Differentially methylated (DM) CpGs between these two populations of nuclei were located in retrotransposons. These FOS DM CpGs were hypomethylated/unmethylated in FOS- cells, indicating that the epiallelic composition of this population is biased towards the unmethylated epiallele. In contrast, all FOS DM CpGs were hypermethylated or fully methylated in FOS+ cells, indicating that the epiallelic composition of recruited cells is heavily biased toward the methylated epiallele. Additionally, we report that DM CpGs were located mostly in promotors and 3'UTR of retrotransposons. Since 5'UTR and independently, 3'UTR, sequences are both abundantly expressed in mammalian cells, and because methylation is inversely correlated with transcription, our data implicate a role of DNA methylation in retrotransposons in transcriptional regulation that promotes recruitment to a coding ensemble. Indeed, we observed the upregulation of synaptic- and neurotransmission-

related genes from a relatively low baseline in FOS- cells to a higher level in FOS+ cells. Together, our data suggest a DNA methylation dependent regulatory mechanism of retrotransposons that, via the reorganization of gene expression, may determine neuronal eligibility to a coding ensemble.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 659.02

Topic: H.08. Learning and Memory

Title: DKK2 regulates hippocampal adult neurogenesis by modulating WNT signaling

Authors: *W. SONG, S. YOON, S. OH, Y. KIM, M.-H. KIM;
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Abstract: WNT signaling plays a pivotal role in normal brain development and function. Dickkopf-related protein 2 (DKK2), a member of the DKK family (DKK1−4) proteins, modulates WNT signaling either positively or negatively in a tissue-dependent manner through the interaction with WNT co-receptors LRP5/6. However, the role of DKK2 in the central nervous system is unknown. Here we show that DKK2 affects adult neurogenesis in the hippocampus by suppressing WNT signaling. Genetic disruption of DKK2 in mice resulted in enhanced β-catenin expression, JNK phosphorylation, and GSK3β phosphorylation in the hippocampus. Incubation of mouse hippocampal slices with recombinant human DKK2 proteins inhibited Wnt3a- or Wnt5a-mediated β-catenin dephosphorylation and JNK phosphorylation, indicating that DKK2 negatively regulates WNT signaling in the hippocampus. DKK2-mutant mice exhibited fewer newborn neurons in the dentate gyrus subfield and impaired performance during the contextual pattern separation test. These attenuated neurogenesis and impaired pattern separation in DKK2-mutant mice were reversed by chronic inhibition of JNK signaling. Collectively, these results suggest that DKK2 enhances hippocampal neurogenesis through the negative regulation of WNT-JNK signaling.

Disclosures: **W. Song:** None. **S. Yoon:** None. **S. Oh:** None. **Y. Kim:** None. **M. Kim:** None.

Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

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Program #/Poster #: 659.03

Topic: H.08. Learning and Memory

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Title: Neurotrophin-3 from the hippocampal dentate gyrus supports mossy fiber-CA3 synaptic transmission and memory

Authors: *J. TAN, H. XU, G.-Y. LIAO, J. AN, B. XU;
UF Scripps Biomed. Res., Univ. of Florida, Jupiter, FL

Abstract: Neurotrophins include nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4/5. BDNF is widely expressed in the adult brain and plays a crucial role in neuronal survival, synaptic plasticity, learning and memory, and the control of mood and feeding behaviors. NT3 also is expressed in the adult brain, but in much fewer brain regions including the dentate gyrus, hippocampal CA2 region, SN and VTA; however, the role of NT3 in the adult brain has not been determined. In this study, we employed a Pomc-Cre transgene, which expresses Cre in dentate gyrus granule cells in the hippocampus, to selectively delete NT3-encoding *Ntf3* gene in the dentate gyrus (Ntf3-cKO). Deletion of Ntf3 in the dentate gyrus didn't affect neuronal survival in the hippocampus. We conducted a series of affective- and memory-related behavioral assays. Ntf3-cKO mice displayed elevated anxious level, impaired contextual fear memory, impaired water-maze spatial memory, and nest building behaviors. As the synaptic structure and function is the basis of an organism's behaviors. We analyzed synaptic transmission at the mossy fiber to CA3 pyramidal neuron pathway (MF-CA3) by ex vivo electrophysiological recordings. Ntf3 deletion in the dentate gyrus impaired basal transmission by reducing AMPAR-mediated excitatory postsynaptic currents (EPSCs) and decreased the amplitude of miniature EPSCs at MF-CA3 synapses without affecting long-term potentiation, paired pulse facilitation, and intrinsic excitability of CA3 pyramidal neurons. By using Thy1-GFP to visualize dendritic structures, we found that Ntf3-cKO mice had fewer and smaller thorny excrescences on proximal apical dendrites of CA3 neurons. Immunostaining revealed that GluR1 expression level was reduced in the CA3 proximal apical dendritic area but not distal apical dendritic area in Ntf3-cKO mice. Thus, our study indicates that NT3 in the dentate gyrus is crucial for synaptic transmission and hippocampal-dependent memory.

Disclosures: **J. Tan:** None. **H. Xu:** None. **G. Liao:** None. **J. An:** None. **B. Xu:** None.

Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

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Program #/Poster #: 659.04

Topic: H.08. Learning and Memory

Support: American Heart Association Predoctoral Fellowship 902999

Title: The effects of BMP signaling on adult-born dentate granule neuron circuits and morphologies

Authors: *Y.-H. TSAI, E. TUNC-OZCAN, C.-Y. PENG, J. A. KESSLER;
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Abstract: Ongoing neurogenesis in the adult hippocampus generates newborn dentate granule cells (GC) that functionally integrate into the existing circuitry and contribute to hippocampus-dependent behaviors. We had previously demonstrated that activation of Bone morphogenetic protein (BMP) signaling negatively regulates adult neurogenesis, and is associated with reduced performance in hippocampal-dependent cognition. However, our recent studies that utilized chemogenetic modulation of neuronal activity in newborn neurons suggest that hippocampal-dependent cognitive behavior can be modified without altering the number of newborn GC. We therefore hypothesized that BMP signaling may alter the functional connectivity of adult-born granule neurons by affecting the morphological maturation and circuit integration, leading to changes in hippocampus-dependent behaviors. Lentiviral constructs expressing either noggin (a BMP inhibitor) or shRNA-noggin were injected into the dentate gyrus (DG) of 8-10 week-old mice to inhibit or enhance BMP signaling specifically in the neurogenic niche. We analyzed the effects of BMP signaling on morphological maturation of the retrovirus-GFP labeled adult-born GCs and found that BMP4 overexpression significantly reduces dendritic length and complexity, while noggin overexpression led to more complex dendritic arborization in newborn GC. Presynaptic connectivity of adult-born GCs with and without BMP signaling inhibition using rabies virus-retrograde tracing will also be presented. Our data suggest that in addition to its roles in regulating stem cell proliferation and fate commitment, BMP signaling affects the dendritic development of adult-born GC in the hippocampus.

Disclosures: **Y. Tsai:** None. **E. Tunc-Ozcan:** None. **C. Peng:** None. **J.A. Kessler:** None.

Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 659.05

Topic: H.08. Learning and Memory

Title: Transcriptional profile of dorsal and ventral regions of the Mus musculus hippocampus under different housing conditions and their association with plasticity process.

Authors: *H. VILLACIS LOZANO[1], M. SALGADO ALBARRÁN[3], P. SALCEDO TELLO[2], R. GONZÁLEZ BARRIOS[4], K. R. GUZMÁN RAMOS[2];
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Abstract: Learning and memory are vital cognitive processes for organisms allowing them to adapt to the changing environment. The rodents' hippocampus has a critical function in the integration of spatial information through plastic changes in its dorsal and ventral segments, especially when animals are exposed to enriched environments, displaying synaptic long-term changes compared to conspecifics living in standard housing conditions. The underlying mechanisms to understand the beneficial effects of environmental enrichment on cognition are still under study and the analysis of transcriptional profiles has opened up new dimensions to identify the key contributing factors. In this study, we identified differentially expressed genes within the dorsal and ventral regions of the hippocampus of *Mus musculus* that experienced different housing conditions during 2 weeks. According to the results obtained, an underexpression is observed in the *Ccdc107* gene, which has commonly been found overexpressed in models with alterations in behavior and cognitive deficits. Moreover, *Cep* is another gene whose *knokdown* has been associated with deficits in learning and memory, however, in our results an overexpression was observed. These findings suggest a neuroprotective effect or rescue processes to cognitive deterioration as a result of accommodation in housing conditions with environmental enrichment. Since housing conditions are determinants of health, our findings have important clinical implications for the hippocampus and disorders that affect cognitive reserve.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 659.06

Topic: H.08. Learning and Memory

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National Institute of General Medical Sciences

Title: Cognitive Impairment and Reduced Dendritic Spine Density after Chronic Chemogenetic Inhibition of Somatostatin-Positive Interneurons in the Dentate Hilus

Authors: *J. LYU[1,2], R. NAGARAJAN[2], M. KAMBALI[2], M. WANG[1,2], U. RUDOLPH[2]; [1]Neurosci. Program, [2]Dept. of Comparative Biosci., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: One of the major hallmarks of cognitive aging is impaired hippocampal function. In aged rodents, hyperactivity of dentate gyrus (DG) granule cells and CA3 pyramidal cells and hypoactivity of CA1 pyramidal cells have been reported, and a reduction of somatostatin-positive interneurons (Sst+ IN) in the DG hilus correlates with cognitive impairment. The neurobiological factors underlying cognitive dysfunction in aging individuals remain unknown.

We propose that chronic inhibition of Sst+ INs in the the DG hilus is sufficient to cause defined learning and memory deficits. We developed a chemogenetic mouse model by stereotaxically injecting the AAV vector hM4D(Gi) bilaterally into the DG hilus of Sst-Cre mice. The control groups were injected with a vector lacking hM4D(Gi). Clozapine was administered to activate hM4D(Gi) and thus inhibit hilar Sst+ INs. The chronic chemogenetic inhibition (CCI) group received clozapine (0.1mg/kg/day) in the drinking water for 21 days before the first behavioral experiment and throughout testing; the acute chemogenetic inhibition (ACI) group was administered clozapine (0.1mg/kg i.p.) daily 30 minutes before behavioral experiments. We found that CCI increased the number of c-Fos+ neurons only in the DG hilus while ACI caused increased c-Fos+ neurons in the whole DG region. In contrast, SST expression was increased in DG and expression of Iba-1, a marker of microglial activation was increased throughout the hippocampus after CCI but not after ACI. We found reduced dendritic complexity of granular neurons in the DG only after CCI as evidenced by the decreased number of dendritic branches and the decreased total dendritic length. In contrast, reduced spine density was observed in DG and CA1 both after CCI and ACI. CCI resulted in a reduced number of mushrooms spines, which are associated with long-term memory storage. Behaviorally, CCI resulted in a decreased recognition index in the novel object recognition test, and an increased path length and increased latency to find the hidden platform in the water maze, both for learning and reversal learning. Our data suggest a causal relationship between a chronic loss of activity of Sst+ INs in the DG hilus and cognitive dysfunction, potentially mediated by microglial activation, reduced dendritic complexity, and a reduced density of mushroom spines. CCI but not ACI thus mimics at least some molecular and behavioral features of hippocampal aging, which itself is a long-lasting process. CCI of DG hilar Sst+ INs may thus be further evaluated as an experimental model of aging-related processes in the hippocampus.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

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Program #/Poster #: 659.07

Topic: H.08. Learning and Memory

Title: Hippocampal adult-born neurons activity during a traumatic event modulates the formation of PTSD-like memory in mice

Authors: *M.-L. KACI[1], J. L. BEROS[3], E.-G. DUCOURNEAU[2], F. FARRUGIA[1], N. D. ABROUS[1], A. DESMEDT[2], M. KOEHL[1];
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Abstract: Post-Traumatic Stress Disorder (PTSD) is a neuropsychiatric disease that develops in some individuals after exposure to a traumatic event. This pathology is classically associated with memory disturbances, where both an emotional hypermnesia for some salient cues present during the traumatic event and an amnesia for the surrounding context coexist. Furthermore, it has been recently suggested that this contextual amnesia could be at the core of PTSD memory impairments. Adult-born neurons (abN) formed in the dentate gyrus of the hippocampus are involved in contextual memory encoding, and could play a key role in PTSD-like memory formation after a traumatic event. Hence, we hypothesized that activating or inhibiting abN could respectively prevent or induce the formation of PTSD-like memory. Using a mouse model that recapitulates the core memory disturbances associated with PTSD and that allows to distinguish between normal and traumatic (PTSD-like) fear memory, we tested this hypothesis. Specifically, we used a viral approach combined with optogenetics to target and activate or inhibit 6-weeks old newborn neurons when the mice were exposed to the traumatic event (electric footshocks associated with corticosterone injection) in a specific context. We then measured the effects of these modulations on freezing behavior when the mice were re-exposed to the conditioning context or to a neutral context in the presence of a cue (tone) that was present during conditioning but that was not predictive of the shock. Our results show that activating hippocampal newborn neurons allows the formation of a normal fear memory in mice that otherwise develop a PTSD-like memory (i.e., amnesia for the traumatic context and abnormal hypermnesia for the tone in a safe context). On the contrary, we also show that inhibiting abN induces the formation of PTSD-like memory in mice that would otherwise exhibit a normal fear memory. Altogether, our results indicate that abN activity during a traumatic event is associated with the formation of either PTSD-like or normal fear memory thereafter, highlighting the relevance of focusing on these neurons to get a better insight into the physiopathology of PTSD.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

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Topic: H.08. Learning and Memory

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2016 LARC-IBRO PROLAB

Title: Dentate Gyrus Somatostatin Cells are Required for Contextual Discrimination During Episodic Memory Encoding

Authors: *C. MORALES[1,2], J. F. MORICI[3], N. ESPINOSA[1], A. SACSON[3], A. LARA-VASQUEZ[1], M. A. GARCÍA-PÉREZ[4,5], P. A. BEKINSCHTEIN[3], N. V. WEISSTAUB[3], P. FUENTEALBA[1];
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Abstract: Memory systems ought to store and discriminate representations of similar experiences in order to efficiently guide future decisions. This problem is solved by pattern separation, implemented in the dentate gyrus (DG) by granule cells to support episodic memory formation. Pattern separation is enabled by tonic inhibitory bombardment generated by multiple GABAergic cell populations that strictly maintain low activity levels in granule cells. Somatostatin-expressing cells are one of those interneuron populations, selectively targeting the distal dendrites of granule cells, where cortical multimodal information reaches the DG. Nonetheless, somatostatin cells have very low connection probability and synaptic efficacy with both granule cells and other interneuron types. Hence, the role of somatostatin cells in DG circuitry, particularly in the context of pattern separation, remains uncertain. Here, by using optogenetic stimulation and behavioral tasks in mice, we demonstrate that somatostatin cells are required for the acquisition of both contextual and spatial overlapping memories.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

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Program #/Poster #: 659.09

Topic: H.08. Learning and Memory

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Title: Maladaptation of dentate gyrus mossy cells mediates contextual discrimation deficit after traumatic stress

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Abstract: Overgeneralization of contextual fear is a maladaptive response to traumatic stress and is associated with the inability to discriminate between threat and safety contexts, which is a hallmark feature of post-traumatic stress disorder (PTSD). However, neural mechanism underlying the overgeneralization of contextual fear in PTSD remains elusive. Here, we show traumatic stress-induced inhibition of dentate gyrus mossy cells (MCs) is responsible for fear overgeneralization. In the learned helplessness (LH) model of PTSD, we find that contextual fear discrimination (CFD) is impaired in stress-susceptible mice, but not in resilient and non-stressed control mice. Based c-Fos immunoreactivity, we find MCs in the dorsal hippocampus are suppressed after repeated exposure to traumatic stress, in susceptible mice. In naïve mice, we find MC activation induced by contextual stimuli drives contextual discrimination through indirect inhibition of dentate granule cells via parvalbumin-positive basket cells, across the long axis of the dentate gyrus. Interestingly, chemogenetic suppression of MCs debilitates intact contextual discrimination in resilient mice. Inversely, chemogenetic activation of MCs is sufficient to restore such cognitive deficit in the susceptible mice. Collectively, these findings indicate that maladaptive changes of MCs after traumatic stress are a substantial mechanism underlying fear overgeneralization with contextual discrimination deficit, suggesting a novel target for advanced therapeutics in PTSD.

Disclosures: M. Jeong: None. **Y. Oh:** None.

Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

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Topic: H.08. Learning and Memory

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Title: Remodeling of CA3 spatial maps by optogenetic activation of immature adult-born neurons

Authors: *M. MUGNAINI, M. F. TRINCHERO, A. F. SCHINDER, E. KROPFF CAUSA, V. C. PIATTI;
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Abstract: Adult hippocampal circuits undergo extensive remodeling by means of activity-dependent synaptic modification and by the generation of new dentate granule cells. While plasticity is fundamental for basic hippocampal functions such as learning, memory and spatial processing, the specific contributions of the distinct mechanisms of circuit modification remain unclear. To investigate the role of adult-born granule cells (aGCs) in spatial processing, we optogenetically stimulated cohorts of aGCs at 4 (young) or 8 weeks of age (mature) and recorded CA3 neural activity while mice freely foraged in an open field environment. Activation of young

(but not mature) aGCs resulted in remapping of a substantial proportion of CA3 place cells despite the fact that they evoked CA3 activity in rare cases. Repetition of the protocol on subsequent days failed to induce further remapping, but a sharp increase in evoked activity similar to mature aGC levels was observed. These findings suggest that immature aGCs bear unique transient capabilities for synaptic transmission and spatial processing, granting them a potential for activity-dependent modification of CA3 spatial maps that decays with functional maturation.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Title: Recovering object-location memories after sleep deprivation-induced amnesia

Authors: *Y. G. BOLSIUS[1], P. R. HECKMAN[2], C. PARACIANI[1], F. RAVEN[3], E. L. MEIJER[1], M. J. KAS[1], S. RAMIREZ[4], P. MEERLO[1], R. HAVEKES[1];
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Abstract: Sleep deprivation is a common problem in our modern 24/7 society. Loss of sleep negatively impacts brain function and in particular cognitive processes that require the hippocampus. In fact, a brief period of sleep deprivation immediately after a hippocampal learning trial leads to memory deficits. It is unclear, however, whether sleep deprivation leads to a loss of information or merely a suboptimal storage of information that is then difficult to retrieve.
In the current study, we addressed this question using memory engram labeling techniques in combination with optogenetics. Male c-fos tTA mice were injected with a TRE-ChR2-mCherry virus in the dentate gyrus, allowing us to specifically tag and reactivate hippocampal memory engram cells. To assess the impact of sleep deprivation on spatial memory processes, we used the object-location memory task with 6 hours of sleep deprivation immediately after the training trial. In line with previous work, we found that sleep deprivation hampered the detection of spatial novelty during a test trial next day (*i.e.*, a relocated object). However, optogenetic activation of the memory engram preceding the test session resulted in a successful detection of the spatial novelty. This observation suggests that sleep deprivation does not lead to the loss of

information, but instead leads to the suboptimal storage of information that cannot be retrieved without optogenetic stimulation. As a next step, we investigated whether these deficits in memory retrievability could also be reversed by treatment with the clinically-approved phosphodiesterase 4 (PDE4) inhibitor roflumilast. Systemic treatment with roflumilast preceding the test session rescued the sleep deprivation-induced memory deficits. When engram activation and roflumilast treatment were combined three days following training and subsequent sleep deprivation, it resulted in a more long-lasting memory trace that allowed for natural retrieval several days later.
Our studies demonstrate that sleep deprivation does not necessarily cause memory loss, but instead may lead to the suboptimal storage of information that is difficult to retrieve. We also provide proof of principle that these suboptimally stored memories can be made accessible again far beyond the learning and sleep deprivation episode and that the clinically-approved PDE4 inhibitor roflumilast may be used to successfully retrieve information thought to be lost.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

Location: SDCC Halls B-H

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Program #/Poster #: 659.12

Topic: H.08. Learning and Memory

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Title: The development of memory specificity in the postnatal rat hippocampus

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Abstract: Episodic memory, or memory for events, is a late-emerging trait in human development. Prior to developing episodic memory, humans experience infantile generalization, an inability to recall specific events, or to correctly place events in their spatial-temporal context (Nelson and Gruendel, 1981). This is thought to occur due to a failure in pattern separation, defined as the brain's ability to reduce the overlap between neural representations of similar sensory inputs, to produce separate memories (Keresztes et al., 2018; Ramsaran et al., 2019). A prominent hypothesis states that pattern separation in adults occurs in the dentate gyrus (DG), a sub-region of the hippocampus (Marr, 1971). The late maturation of DG granule cells could

therefore be one cause of infantile generalization. However, although granule cells are the primary excitatory cell in the DG they fire sparsely and the less numerous but higher firing rate mossy cells, are also thought to be implicated in the process of pattern separation. Discriminating the firing of these two cell types has previously posed a challenge for in vivo studies of the DG. The present work characterizes cells from the developing DG, using cell firing properties and anatomical position to separate mossy and granule cell firing (Goodsmith et al., 2019; Senzai and Buszaki, 2017). We investigated the pattern separation properties of DG neurons by exposing lister hooded rats (N=14) to spatial contexts of differing similarities, whilst performing in vivo electrophysiological recordings using tetrodes and silicon probes across the age range that corresponds to the emergence of hippocampal memory (P16-P32). Preliminary results show that pattern separation on the basis of firing rate is impaired in young animals, in granule cells though not mossy cells. The data presented here characterizes the changing properties of different cell types in the dentate gyrus and contributes to understanding their role in pattern separation as it emerges in the postnatal rat.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

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Topic: H.08. Learning and Memory

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Title: Double dissociation between the enclosed and exposed blade of the Dentate Gyrus (suprapyramidal vs infrapyramidal) in terms of spatial and non-spatial information processing

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Abstract: One of the most influential models of episodic memory posits that spatial and non-spatial information segregated at the cortical level are ultimately integrated at the level of the hippocampus. Recent anatomical and IEG imaging studies raised, however, the possibility that spatial and non-spatial information could also be processed in a segregated manner along the proximodistal axis of the hippocampus by distinct subnetworks (Beer and Vavra et al, 2018; Flasbeck et al, 2018; Nakamura et al, 2013). This would especially be the case when one dimension of the memory representation is overly relevant compared to the other and the integration of the less/non-relevant information is dispensable. The ‘spatial’ subnetwork includes the enclosed blade (suprapyramidal) of the Dentate Gyrus (DG), the distal part of CA3 and the proximal part of CA1 (both close to CA2) and the medial entorhinal cortex. The ‘non-spatial’ subnetwork includes the exposed blade (infrapyramidal) of the DG, the proximal part of CA3 (close to the DG), the distal part of CA1 (close to the subiculum) and the lateral entorhinal

cortex. Functional evidence for the existence of these subnetworks are, however, only available for CA1 and CA3 to date. Hence, it is still unclear whether spatial and non-spatial information are processed differentially by the enclosed and exposed blades of the DG. Here, we show that preferential optogenetic inhibition (ArchT) of the enclosed blade yields a detrimental effect on memory performance in a murine object-location task taxing DG function (Van Hagen et al, 2015), while performance on the non-spatial version of this task (Oule et al, 2021) remains unaffected. In contrast, preliminary data suggest that inhibiting cell firing in the exposed blade of the DG affects discrimination in the non-spatial version of the task. Additionally, the effect of optogenetic inhibition of the enclosed blade in the spatial version of the task on the recruitment of other areas of the subnetwork is currently investigated using Arc imaging. This result adds to previous reports of a functional segregation of the blades of the DG in terms of inputs/outputs areas, neurogenesis and sensitivity to stress and suggests that the enclosed and the exposed blade of the DG are part of distinct hippocampal subnetworks preferentially processing spatial and non-spatial information, respectively.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Title: Dopamine D2 receptors in hilar mossy cells modulate spatial memory, anxiety-like behavior, and epileptic activity

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Abstract: Expression from the dopamine D2 receptor (D2R) promoter is a hallmark feature of dentate gyrus hilar mossy cells (MCs), but the physiologic function of the MC D2R is unknown. MCs are uniquely positioned to shape dentate gyrus function and have been implicated in several learning and disease processes, but the mechanisms underlying their involvement are not well-understood. Dopaminergic fibers are present in the dentate gyrus, and hippocampal dopaminergic signaling is involved in processes and pathological states now associated with MCs. These include spatial memory, anxiety, and epilepsy. We hypothesized that D2Rs modulate key aspects of MC function in memory and disease. To address this possibility, we

assessed potential behavioral impacts of genetic MC D2R removal. We injected 3- to 4-month-old floxed-Drd2 mice with AAV5-CamKII-mCherry-Cre or AAV5-CaMKII-mCherry to generate MC D2R conditional knockout (cKO) and control mice, respectively, for behavioral testing. Results indicate that conditionally knocking out D2Rs from MCs impairs spatial memory, promotes anxiety-like behavior, and increases seizure severity and susceptibility. We are currently investigating the role of MC D2R signaling in dentate gyrus physiology through electrophysiology and immunohistochemistry (e.g., c-Fos labeling) to better understand the cellular mechanisms underlying the observed behavioral effects. Determining the function of MC D2Rs can lead to better understanding of MCs' roles in normal physiology and disease states.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Program #/Poster #: 659.15

Topic: H.08. Learning and Memory

Support: R01 NS104776

Title: Nrem-targeted activation of medial septal cholinergic input to hippocampus disrupts consolidation of hippocampal-dependent memories

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Molecular, Cell. & Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Long-term memory consolidation is an indispensable process, which can be obstructed by post-learning sleep disturbances. As the brain transitions from wakefulness to non-rapid eye movement (NREM) sleep to REM sleep, the hippocampus undergoes changes in both network activity and levels of neurotransmitter release. For example, acetylcholine (ACh) release in the hippocampus is lowest during NREM sleep and highest during REM sleep. Recent work from our lab has shown that chemogenetic activation of medial septum (MS) ACh input to the hippocampus, following contextual fear conditioning (CFC), is sufficient to decrease network activity in the dentate gyrus (DG) and disrupt contextual fear memory consolidation. In contrast, chemogenetic inhibition of these inputs promotes DG activity and fear memory consolidation. Together, this suggests that reduced levels of MS ACh release during NREM sleep could be required for hippocampal memory consolidation. To test this, we measured the effects of NREM-specific optogenetic activation of MS ACh neurons on behavioral measures of memory consolidation and *in vivo* electrophysiology. Adult ChAT-IRES-Cre mice were transduced with either a Cre-dependent control vector (YFP) or ChR2-EYFP delivered via AAV to the MS. An optical fiber was implanted above the MS, along with EEG electrodes placed bilaterally to record hippocampal network activity during sleep states. Mice were trained on one of two different memory tasks - object-location memory (OLM) and CFC, after which they were allowed *ad lib*

sleep, with or without optogenetic stimulation of MS ACh neurons over the first 6 h of NREM sleep. Memory for each task was evaluated 24 h later. Ninety minutes after recall of the final memory task, mice were perfused for quantitative immunohistochemistry of recall-associated hippocampal immediate-early gene (IEG) expression. We found that NREM-specific activation of MS ACh neurons disrupted memory consolidation in both the OLM and CFC tasks. This could suggest that the suppression of ACh release in the hippocampus during NREM sleep is necessary for proper hippocampus-mediated memory consolidation. Ongoing studies are assessing how the disruptive effects of ACh release during NREM on consolidation relate to activation patterns of excitatory and inhibitory neurons in the hippocampal DG network during recall.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Title: GIRK channel activation improves sleep phenotypes and memory consolidation in a mouse model of Fragile X syndrome

Authors: *J. D. MARTINEZ[1], L. G. WILSON[1], K. G. PETERSON[1], W. P. BRANCALEONE[1], M. J. DONNELLY[1], D. S. POPKE[1], R. E. PEREZ TREMBLE[1], S. J. ATON[2];
[1]Molecular, Cellular, and Developmental Biol., Univ. of Michigan, Ann Arbor, MI; [2]Univ. of Mchigan, Univ. of Mchigan, Ann Arbor, MI

Abstract: Fragile X syndrome (FXS), associated with loss of Fmr1 protein, is the leading genetic cause of intellectual disability. FXS is also associated with profound sleep abnormalities. Sleep loss itself negatively impacts cognitive function (e.g. memory consolidation) and related synaptic plasticity mechanisms. However, the contribution of sleep loss to impaired cognitive function in FXS is understudied. One untested possibility is that disrupted cognition in FXS is exacerbated by abnormal sleep. Recent data have suggested that sleep is necessary for renormalizing firing rates and regulating excitatory/inhibitory balance in neural circuits involved in cognition, including hippocampus and neocortex. We hypothesized that disruption of these sleep-dependent mechanisms disrupt information processing in these circuits, to impair cognitive functions such as memory consolidation. We examined whether ML297, a novel hypnotic acting on G-protein-activated inward-rectifying potassium (GIRK) channels, could promote normal

sleep and rescue deficits in memory consolidation in male mice lacking Fmr1 (*Fmr1-/y*). Using multi-day EEG recordings, we show that 4-5 month old *Fmr1-/y* mice have reduced overall NREM sleep, fragmented NREM sleep architecture, and altered NREM delta power compared to wild-type littermates. Activation of GIRK channels via ML297 administration normalizes NREM sleep amounts, normalizes delta oscillations, and rescues NREM fragmentation in *Fmr1-/y* mice. We assessed sleep-dependent contextual fear memory (CFM) consolidation in *Fmr1-/y* mice. We found that deficits in CFM consolidation in *Fmr1-/y* mice can be rescued with post-conditioning GIRK activation via ML297 in a sleep-dependent manner. Lastly, we find that ML297 administration following conditioning results in altered patterns of cFos expression in the dorsal hippocampus during CFM recall, with differential effects on principal (excitatory) neurons and parvalbumin-expressing interneurons. These studies provide a comprehensive examination of the impact of sleep and sleep loss on neurophysiological, neuroanatomical, and behavioral phenotypes of FXS, and support a role for sleep as a therapeutic target for improving health and cognitive outcomes in FXS.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Topic: H.08. Learning and Memory

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Title: Differential effects of learning and sleep deprivation on gene expression and biological pathways in different dorsal hippocampal subregions

Authors: *L. WANG, L. PARK, A. VEGA MEDINA, F. RAVEN, J. MARTINEZ, W. WU, D. KING, S. ATON;
Univ. of Michigan, Univ. of Michigan, Ann Arbor, MI

Abstract: In both animal models and human subjects, studies have shown that post-learning sleep loss impairs performance on dorsal hippocampus-dependent memory tasks. For example, in mice, 5 to 6 hours of sleep deprivation (SD) following contextual fear conditioning (CFC) may disrupt contextual fear memory consolidation. Furthermore, sleep deprivation has been shown to affect hippocampal gene expression and biochemical pathways involved in learning and memory consolidation. However, it is unclear how sleep and SD affect the transcriptome across various hippocampal subregions (e.g. CA1, DG, and CA3), and how this changes as a function of prior learning. Using spatial transcriptomics, we show, for the first time, how hippocampus-dependent learning (CFC), sleep, and SD alter mRNA expression and biological

pathways in the mouse hippocampal CA1, DG, and CA3. We find that transcripts regulated by either prior learning or SD are largely different between these subregions. Moreover, mRNAs differentially affected by sleep vs. SD are involved in cellular pathways involved in information storage in the brain, including glutamatergic synapse, circadian entrainment, and retrograde endocannabinoid signaling. These data provide new insight into how hippocampal memory processing could be disrupted by post-learning sleep deprivation.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Topic: H.08. Learning and Memory

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Title: Differential gating of dorsal hippocampus-dependent memory processing stages by somatostatin- and parvalbumin-expressing interneurons

Authors: *F. RAVEN, A. A. VANKAMPEN, S. J. ATON;
Molecular, Cellular, and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Memory encoding, consolidation, and recall are all thought to rely on the activation of neurons across multiple brain regions. While recent studies have focused on the selective activation of excitatory (i.e., glutamatergic) engram neurons during hippocampal memory processing, inhibitory (i.e., GABAergic) interneurons likely play a role in modulating memory processing. Here we tested the roles of parvalbumin-expressing (PV+) and somatostatin-expressing (SST+) interneurons in modulating various stages of memory processing, including encoding, consolidation, and recall. Adult male and female SST-IRES-Cre and PV-IRES-Cre mice underwent AAV-mediated transduction to express either the activating DREADD hM3Dq-mCherry, or mCherry alone, in a Cre-dependent manner within dorsal hippocampus. Hippocampus-dependent memory processing was assessed at the beginning of the light phase using the object-location memory (OLM) paradigm. Clozapine-N-oxide was administered to all mice i.p. at different phases: either 30 min before OLM training (to test for effects on interneuron activation on memory encoding), or directly after the training (to test for effects on OLM consolidation), or 30 min prior to OLM testing 24 h after training (to test for effects on retrieval). Preliminary data suggest that activation of hippocampal SST+ interneurons significantly impairs OLM retrieval, but not memory encoding or consolidation (in contrast to prior reports on consolidation of other hippocampus-dependent memory types, such as contextual fear memory). Interneuron populations can be differentially affected by sleep and sleep loss, and can

differentially regulate activity in brain circuits. Thus, understanding the contributions of these populations in gating various stages of memory processing will have important implications for neurological disorders where their function is altered.

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Poster

659. Dentate Gyrus: Molecular and Circuit Mechanisms of Encoding

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Title: Increasing adult hippocampal neurogenesis enhances social recognition memory by recruiting a feedforward inhibitory circuit

Authors: *A. CHUNG[1,2], S. MILLER[1,2], M. GHOSH[3], O. J. AHMED[3,4], A. SAHAY[1,2,5,6]; [1]Ctr. for Regenerative Med., MGH, Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]Dept. of Psychology, [4]Dept. of Biomed. Engin., Univ. of Michigan, Ann Arbor, MI; [5]BROAD Inst. of Harvard and MIT, Cambridge, MA; [6]Harvard Stem Cell Inst., Cambridge, MA

Abstract: Social recognition memory is critical for adaptive social behavior. The dentate gyrus is host to generation of new neurons, dentate granule cells, throughout life. Adult-born dentate granule cells (abDGCs) contribute to spatial or contextual memory formation, discrimination, and consolidation. In contrast, the neural circuit mechanisms by which adult-born dentate granule cells (abDGCs) contribute to social recognition memory are poorly understood. Here, we show that genetically increasing adult hippocampal neurogenesis improves social memory discrimination in a social memory interference task. Genetic expansion of a discrete population of age-matched population of abDGCs increased Parvalbumin inhibitory interneurons (PV IN) perisomatic contacts on CA2 pyramidal neurons. This enhanced PV IN structural plasticity was accompanied by an increase in functional inhibitory synaptic inputs onto CA2 pyramidal neurons. To understand how these changes in feed-forward inhibition in dentate gyrus (DG)-CA2 circuit affect network properties, we recorded local field potentials in CA1 during sleep and in the social memory task. Mice with genetically expanded population of age-matched abDGCs exhibited an increase in CA1 sharp-wave ripple (SWR) power during sleep and reduced Gamma range (30-100Hz) oscillatory neural activity during non-REM sleep. Social interactions increased the CA1 SWR rate in mice with more age-matched abDGCs relative to the control group. Our

findings begin to define neural circuit and network mechanisms by which new neurons in the adult dentate gyrus contribute to social information processing.

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Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.01

Topic: H.08. Learning and Memory

Title: Development of an Age-Dependent Cognitive Index: Impaired Learning and Disturbances in Circadian time keeping

Authors: *K. DE SOUZA[1], A. POWELL[1], G. C. ALLEN[1], D. EARNEST[2];
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Abstract: Cognitive quantification in preclinical studies is necessary in order to achieve higher translational power from basic studies to clinical observations. Most tests used to characterize learning in rodents require repetitive testing and are useful for demonstrating performance and change over time, but these results cannot be used in correlative experiments. We developed a cognitive index for learning based on previously described scores for strategies used by mice to reach the escape box in the Barnes maze. This single number allows mice to be placed in a continuous scale based on performance that allows for measuring the severity of age-related cognitive impairment. Aged mice (18-24 mo) showed significant deficits when compared to young (4-6 mo) and middle aged (13-14 mo), and the cognitive index significantly correlated to the memory portion of the task (probe).Cognitive changes in aging are often accompanied by pronounced disturbances of circadian timekeeping, especially the sleep-wake cycle. Because the aging mouse cohort showed variability in onset and magnitude of cognitive impairment, we explored the relationship between these cognitive deficits and sleep disturbances during aging in mice. The circadian rhythm of locomotor activity was continuously analyzed for 30-40 days in young, middle-aged and aged mice. Aged mice exhibited significant impairment of cognitive impairment behavior in conjunction with marked alterations in the circadian entrainment of their activity rhythms Data from middle-aged animals showed that unstable patterns of circadian entrainment and increased variability in the daily onsets of activity occur before deficits in learning and memory. Interestingly, we observed a relationship between the extent of cognitive impairment in the Barnes maze and variability in daily onsets of circadian activity in spatially-impaired mice. This data is the foundation of our model to further understand the relationship between age-related changes in circadian rhythms and cognitive impairment, and to probe possible mechanisms of action.

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Poster

660. Timing and Temporal Processing

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Title: The effect of ITI duration and reinforcement delay on rats' midsession reversal performance

Authors: *A. TAVERA-GALICIA[1], C. SANTOS[2], J. BURITICÁ[1];
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Abstract: In the midsession reversal task (MSR), subjects learn a simultaneous simple discrimination with a reversal. During the first half of the session, responses to S1 are reinforced and the responses to S2 are extinguished. In the second half of the session, contingencies are reversed. Typically, rats develop a response pattern resembling a win-stay/lose-shift response strategy using the memory of the outcome of the previous response to determine the next. However, when the past outcomes of behavior are not good predictors of the reinforcement, interval timing seems to be the go-to strategy to solve the task; like pigeons commonly do. This strategy is evident in the tendency to continue to respond to the S1 past the moment of the reversal (perseveration) and, more importantly, to start responding to S2 before the reversal has taken place (anticipation). When the estimation of time elapsed form the begining of the session determines performance, perseverative and anticipatory errors cluster around the moment of the reversal. To assess the determinants in the use of one strategy over the other in seemingly similar situations, we trained six Wistar rats in a MSR task. In a second phase, we doubled the length of the ITI to test the idea that interval timing aids performance when the memory of the outcome of the previous trial is no longer available, and last, we introduced a delay between the response and the reinforcer to explore the effect of weakening the contiguity of response and outcome. Preliminary analysis showed rats rapidly adopted a win-stay/lose-shift strategy I the first phase and remained undisturbed both by the increase on the duration of the ITI and the reinforcement delay. In contrast to pigeons, rats' performance did not show temporal control when the ITI was lengthened. Overall, the MSR task proved to be a suitable preparation to study the competition between two simultaneous discriminative stimuli and interval timing and past outcomes. The present study suggests that rats' working memory allows them to use the memory of past outcomes a predictor of a reinforcement and is considerably robust; even when time between learning episodes (ITI) and between response and reinforcer(delay) is relatively long.

Disclosures: **A. Tavera-Galicia:** None. **C. Santos:** None. **J. Buriticá:** None.

Poster

660. Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 660.03

Topic: H.08. Learning and Memory

Support: OPUS 2019/39/B/HS6/04389

Title: Rodents monitor their error in self-generated duration

Authors: *T. KONONOWICZ[1], L. LE BARILLIER[1], V. VAN WASSENHOVE[2], V. DOYERE[3];
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Abstract: When faced with a deadline, individuals' behavior suggests that they represent the mean and the uncertainty of an internal timer to make near-optimal time-dependent decisions. Whether this ability relies on simple trial-and-error adjustments, or whether it involves richer representations, is unknown. Richer representations enable a possibility of error monitoring, that is, the ability for an individual to assess its internal representation of the world and estimate discrepancy in the absence of external feedback. While rodents show timing behavior, whether they can represent and report temporal errors in their own produced duration on a single trial basis is unknown. We designed a novel paradigm requiring rats to produce a target time interval to receive a reward in a given location depending on the magnitude of their timing errors. During the test-trials, rats had to choose a port corresponding to the error magnitude of their just-produced duration to receive a reward. High choice accuracy demonstrates that rats kept track of the values of the timing variables on which they based their decision. Additionally, they kept a representation of the mapping between those timing values and the target value, as well as the history of the reinforcements. The results show for the first time that rats track their own timing errors, deepening our understanding of error monitoring abilities in rodents and the richness of their representation of elapsed time. This paradigm offers a new way to research the neural architecture underlying the ability of neural systems to monitor their own computations. We further use this paradigm in conjunction with muscimol injections to test whether Orbitofrontal Cortex and Cingulate Cortex are involved in temporal error monitoring.

Disclosures: T. Kononowicz: None. **L. Le Barillier:** None. **V. van Wassenhove:** None. **V. Doyere:** None.

Poster

660. Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 660.04

Topic: H.08. Learning and Memory

Support: KAKENHI 19H01775
KAKENHI 22K03200

Title: Behavioural contrast in the temporal domain in mice

Authors: *Y. KOSAKI, T. HU;
Waseda Univ., Tokyo, Japan

Abstract: Our perception of the environment is often relative rather than absolute. In the present study, we explored whether perception of time shows any relativity in mice. More specifically, we asked if interval timing for one duration would be affected by concurrent training on another interval timing, using a multiple-schedule peak procedure. C57BL/6J mice were trained to lever press for sucrose solution under a multiple FI-FI schedule, with different FI schedules assigned to two levers. In Experiment 1, half the animals were trained on a Multi FI 15 s - FI 30 s schedule, whereas the other half animals were given training on a Multi FI 30 s - FI 60 s schedule. After a stable FI performance was obtained, peak probe trials were inserted on 20% of the trials each session. The peak-procedure training was continued for 25 sessions. The resultant peak functions exhibited a moderate but systematic left-ward shift for the longer duration lever, whereas no deviation was observed for the shorter duration lever. When the peak timing for the 30-s lever from the two groups was directly compared, the left-ward shift was still significant for the group for which 30 s was the relatively longer of the two intervals to time. Experiment 2 replicated the finding by training mice with either a Multi FI 10 s - FI 40 s schedule or a Multi FI 20 s - FI 30 s schedule; in both groups, systematic left-ward shift of peak timing was observed for the longer duration lever. The left-ward shift for the longer duration observed here indicates that the interval timing generalised from one duration to another, but in an asymmetric manner: from short to long but not long to short durations. This result could imply that the mice continued to perceive the longer duration even longer than it actually was, due to that the response peak for the longer duration consistently appearing earlier; that is, each reinforcement has occurred later than expected. This overestimation of elapsed time was produced by the requirement of timing a shorter interval in the same context. At a more descriptive level, the current results demonstrate a novel behavioural contrast in the temporal domain, and an asymmetry in such a contrast effect.

Disclosures: **Y. Kosaki:** None. **T. Hu:** None.

Poster

660. Timing and Temporal Processing

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Topic: H.08. Learning and Memory

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Title: Local 5-HT signal bi-directionally regulates the coincidence time window of associative learning

Authors: *J. ZENG[1,2,3,4], X. LI[2,3], Z. ZHANGREN[2,3,5], M. LV[2,3], Y. WANG[2,3], K. TAN[2,3], X. XIA[2,3,6], J. WAN[2,3], M. JING[7], Y. YANG[8,9], Y. LI[8,9], Y. LI[2,3,4,6,7];
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Abstract: Temporal coincidence between the conditioned stimulus (CS) and unconditioned stimulus (US) is essential for associative learning across species. Despite its ubiquitous presence, the mechanism that may regulate this time window duration remains unclear yet. Using olfactory associative learning in *Drosophila* as a model, we find that suppressing or promoting serotonin (5-HT) signal could respectively shorten or prolong the coincidence time window of odor-shock associative learning and synaptic plasticity in mushroom body (MB) Kenyon cells (KCs). Capitalizing on GPCR-activation based (GRAB) sensors for 5-HT and acetylcholine (ACh), we characterized the *in vivo* 5-HT dynamics in MB lobes during odor and shock stimulations and further dissected this microcircuit. Interestingly, local KC-released ACh activates nicotinic receptors on the dorsal paired medial (DPM) neuron, and in turn the DPM neuron releases 5-HT to inhibit the ACh signal via the 5-HT1a receptor. Finally, we demonstrated that the DPM-mediated serotonergic feedback circuit is sufficient and necessary to regulate the coincidence time window. This work provides a model for studying the temporal contingency of environmental events and their causal relationship.

Disclosures: J. Zeng: None. **X. Li:** None. **Z. Zhangren:** None. **M. Lv:** None. **Y. Wang:** None. **K. Tan:** None. **X. Xia:** None. **J. Wan:** None. **M. Jing:** None. **Y. Yang:** None. **Y. Li:** None. **Y. Li:** None.

Poster

660. Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 660.06

Topic: H.08. Learning and Memory

Title: Temporal interval learning in a mouse model of Fragile X syndrome

Authors: *S. ALI[1], O. ABU-WISHAH[3], S. POST[4], A. GOEL[2];
[2]Psychology, [1]Univ. of California Riverside, Riverside, CA; [3]Unviersity of California, Riverside, Riverside, CA; [4]Unviersity of California, Riverside, RIVERSIDE, CA

Abstract: Interval timing is a fundamental process that allows estimation and discrimination between different durations of sensory stimuli to guide future predictions. For example, waiting to cross a busy intersection requires determining the duration of sensory stimuli to predict whether the next action should be to go or stop. Impairments in estimating durations of sensory events can contribute to symptoms of Fragile X syndrome (FXS), such as, an inability to predict future events, perseverative actions and deficits in speech perception and production. To delineate the network mechanisms that contribute to deficits in interval timing in *Fmr1* KO mice (a mouse model of FXS), we designed a novel timing task – the temporal interval sensory discrimination (TISD) task. In the TISD task, mice are presented with visual stimuli that differ only in their duration–a long and short interval, and the long interval is associated with a water reward. Our findings show that wild type (WT) and *Fmr1* KO mice learn to discriminate between the durations of visual stimuli and preferentially lick in response to the long duration visual stimuli. Learning in both WT and *Fmr1* KO mice is evident in the distinct licking patterns that differ between the short and long intervals and a discriminability index (d') greater than 2 (number of sessions to reach d' of 2: WT mice 8 sessions; *Fmr1* KO mice 9 sessions). However compared to WT mice, licking patterns in *Fmr1* KO mice are less selective to the preferred stimulus. These results indicate that WT and *Fmr1* KO mice can use information about stimulus durations to guide their decisions, although *Fmr1* KO mice show broader and less refined licking patterns. Our current experiments combine the TISD task and two-photon calcium imaging to examine changes in neural networks of the primary visual cortex (V1). We predict that distinct neural network dynamics in V1 contribute to learning of the interval timing task.

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Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.07

Topic: H.08. Learning and Memory

Support: Scholarship to ME 701506 from CONACyT (México)
CONACyT INFR-281265 to JB

Title: Effects of delayed reinforcement in prospective timing with rats

Authors: *M. EUDAVE-PATIÑO[1], E. ALCALÁ[2], T. CAMPOS-ORDOÑEZ[1], J. BURITICÁ[1]; [1]Ctr. de Estudios e Investigaciones en Comportamiento, Univ. de Guadalajara, Jalisco, Mexico; [2]Dept. of Mathematics and Physics, ITESO, Jalisco, Mexico

Abstract: Several factors affect performance in schedules of reinforcement. In Fixed Interval (FI) schedules some of those factors are the duration of the interval, and the reinforcement value used in the procedure. Delay of reinforcement affects its effectiveness, and some experiments suggest that the delay of reinforcement creates flat generalization gradients around the trained interval and generates a devaluation of reinforcement. This flat gradient means that expectation of reinforcement is noisy (less precise) compared to when immediate reinforcement is used. The explanation of the effect could be that increasing the time interval between the response and the reinforcement may diminish the correlation between both events, and such lower contingency between response and reinforcer could decreases the response rate. The fact that delayed reinforcement creates flat generalization gradients, that time expectation is noisier, still need to be more robustly stablished, that is why in this experiment we used two durations of delay of reinforcement in two groups (10 % and 20 % of the trained interval), each group with eight naïve male Wistar rats, aged four months old at the beginning of the experiment. They were trained in a multiple schedule of two components: at baseline the same FI was scheduled in both components, in the experimental phase one component maintained the same average time to reinforcement (30 s) but with delayed reinforcement while the intervals in the second component were yoked to the first component but with immediate reinforcement. In both phases 10 % of peak trials were presented. We analyzed the fixed intervals using inter-response times (IRTs) durations modelled using loess regression to describe the effect of delay of reinforcement and also analyzed the peak trials. IRTs durations modelled using loess regression changed from long to short at a proportion of the FI and that proportion increased in the delayed component. Longer delays changed the performance to negative accelerated curves instead of the scalloped patterns and disorganized the performance in peak trials at the level that most common analysis such as start-stop analysis could not generate reliable estimates of timing. The delay of reinforcement, its duration, is a crucial factor affecting timing and performance in this prospective timing procedure. The importance to establish how delay of reinforcement affects timing is crucial to understand if delay of reinforcement changes reinforcement value and how it changes the performance in timing especially processes like memory, attention and learning as assumed by models like the Scalar Expectancy Theory (SET) and Learning to Time (LeT).

Disclosures: M. Eudave-Patiño: None. **E. Alcalá:** None. **T. Campos-Ordoñez:** None. **J. Buriticá:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.08

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI Grant Number 22J20401

Title: Precise peak timing behavior favors habitual control: a potential competition between timing and outcome value?

Authors: *T. HU, Y. KOSAKI;
Psychology, Waseda Univ., Tokyo, Japan

Abstract: The current study aimed at understanding the processing of outcome value and outcome timing in instrumental behavior. Four experiments examined the outcome-specific devaluation effect on interval timing performance in rats using a 20-s peak task. In all experiments, rats learned to discriminate two stimuli signaling different outcomes. Prior to testing under extinction, a LiCl-induced taste aversion was established to one of the outcomes. Using a discrete-trial procedure, Experiments 1 and 2 revealed insensitivity to devaluation (i. e., habit) and intact peak timing. The absence of devaluation effect was observed regardless of whether the aversion was conditioned with isotonic (Exp. 1) or hypertonic (Exp. 2) LiCl solution. Training rats in a discriminated free-operant procedure, Experiment 3 observed a devaluation effect, but instead a previously overt response peak diminished. When the temporal control of free operant was facilitated by increasing ITIs in Experiment 4, rats displayed a clear peak responding at expense of losing the devaluation effect. Collectively, timing behavior with a clear response peak consistently showed insensitivity to the devaluation, whereas behavior lacking the precise temporal control revealed its reliance on the current value of that outcome. This pattern of results could suggest that outcome timing competes for control over the behavior with other detailed features of the outcome, including its incentive value; these two learning processes may be in a trade-off relation. Thus, instrumental behavior that is strongly governed by the elapsed time might inevitably become habitual.

Disclosures: T. Hu: None. **Y. Kosaki:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.09

Topic: H.08. Learning and Memory

Support: NSF RI:Small 2008741

Title: Enhancing the computational power of neural networks through plasticity of short-term-synaptic-plasticity

Authors: *S. ZHOU[1], D. V. BUONOMANO[2];
[1]Neurobilogy, Univ. of California, Los Angeles, Los Angeles, CA; [2]UCLA, Los Angeles, CA

Abstract: The principle that long-term changes in synaptic strength are the primary mechanism underlying learning and memory is among the most influential concepts in neuroscience and underpins many of the advances in machine learning. The vast majority of neurocomputational and machine learning models, however, do not incorporate one of the most universal forms of synaptic plasticity: short-term plasticity which refers to the fact that the strength of synapses can facilitate or depress in a use-dependent fashion on the scale of tens of milliseconds to seconds. The few models that do incorporate STP generally assume it is fixed. Although virtually all synapses in the brain exhibit STP, two important questions have not been well addressed: 1, whether STP is synapse-specific, i.e., whether the synapses from the same presynaptic neuron onto postsynaptic neurons of the same class possess the same or distinct set of STP parameters; 2, whether the temporal profile of STP is learned, i.e., is STP governed by learning rules that enhance its contribution to neural computations. Here we first used a dataset from the Allen institute (*Campagnola et al., Science 375, 1144, 2022*), to determine if the temporal profile of STP of synapses from the same presynaptic neuron onto two postsynaptic neurons (of the same neuronal class) was correlated or not. By fitting the EPSP profiles, we found that the correlation of STP parameters between synapses with a shared presynaptic neuron was either nonsignificant or weak suggesting that STP is synapse-specific and that learning rules may be in place to independently govern not only the strength of synapses but the temporal profile of STP as well. To address the potential computational benefits of plasticity of STP, we incorporated STP into a feedforward firing rate neural network and trained it to solve a complex time-dependent task: recognizing Morse code letters. By training the weights and STP parameters we found that networks with the plasticity of STP increase both learning speed and performance compared to networks without STP or with fixed STP. Moreover, spiking network models were also implemented and confirmed the finding. Traditionally, machine learning tasks that require maintaining a temporal history of previous stimuli, are solved either through RNNs or the nonbiological spatialization of time in feed-forward networks. Here we show that networks with STP can solve temporal problems in the absence of recurrent connections or the spatialization of time and that plasticity of STP can significantly enhance the computational power of neural networks, leading to the prediction that there are biological learning rules in place to govern STP.

Disclosures: **S. Zhou:** None. **D.V. Buonomano:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.10

Topic: H.08. Learning and Memory

Support: CONACyT INFR- 281265
CONACyT-1178107

Title: Effects of delayed reinforcement in a Free Operant Psychophysical Procedure in rats

Authors: *G. OCHOA-ZENDEJAS[1], M. EUDAVE-PATIÑO[1], A. TAVERA-GALICIA[1], C. SANTOS[2], J. BURITICÁ[1];
[1]Ctr. de Estudios e Investigaciones en Comportamiento, Univ. de Guadalajara, Guadalajara, Jal., Mexico; [2]Dept. of Psychology, Arizona State Univ., Arizona, AZ

Abstract: Delay of reinforcement affects timing behavior, reducing discriminative control by creating flat generalization gradients around the trained interval, it also increases the Weber fraction and decreases the response rate. Timing research has reported these effects in timing tasks like peak procedure, fixed interval and temporal bisection. However, there are no experiments of the effect of delay of reinforcement in a Free Operant Psychophysical Procedure (FOPP), a task used to study timing behavior. FOPP has been studied in pigeons and rats, typically, at the steady state, two response-rate generalization gradients are obtained, one for each operant. The rate on the S1 starts high and then decreases, whereas the rate on the S2 starts low and then increases. The gradients intersect at a time close to the middle of the trial. The relative response-rate gradient, defined by the proportion of right responses at each trial moment, follows a roughly ogive curve. In summary, subjects move between options following the change in contingencies around half of the trial even though the reinforced responses are unpredictable according to an IV. For this study we used FOPP to study the effect of delay of reinforcement and to establish if the effects that have been reported in the literature in other timing procedures also appear in this procedure. The aim of this study was to observe the effect of the delay of the reinforcement in FOPP. Ten male Wistar rats were used, when they were approximately 120 days old. The rats were food deprived 85% of their weight ad libitum. The sessions consisted of 75 trials with presentation of two-alternatives of response (S1 vs. S2) in which the S1 responses were reinforced during the first half of a 60 s trial, and the responses to S2 were extinguished. For the last half of the trial the contingency was reversed, reinforcement for responding on S2 and extinction in S1. In the first condition the reinforced responses followed two independent Variable Intervals (VI) 60 s. In the second phase, an unsignaled delay in reinforcement of 6 s was implemented and the VI changed to 54 s. Preliminary results showed that animals were sensitive to the delay of reinforcement, response rate decreased, and the psychometric function changed. This result is consistent with previous reports where modifying the response-reinforcement relationship produced more noise in time estimation of the subjects. The delay of reinforcement and its effect on time estimation in this type of procedure should be carefully analyzed to learn more about its effect at the behavioral level and to understand how this could affect processes in the brain like learning and memory of time intervals.

Disclosures: G. Ochoa-Zendejas: None. **M. Eudave-Patiño:** None. **A. Tavera-Galicia:** None. **C. Santos:** None. **J. Buriticá:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.11

Topic: H.08. Learning and Memory

Support: Humboldt Research Fellowship

Title: Neurons in the nidopallium caudolaterale of the carrion crow encode temporal information.

Authors: *M. JOHNSTON, M. E. KIRSCHHOCK, A. NIEDER;
Univ. of Tübingen, Univ. of Tuebingen, Tübingen, Germany

Abstract: Flexibly monitoring time over a period of seconds or minutes, an ability known as "interval timing", is critical for many goal-directed behaviours in both humans and non-human species, e.g., optimal foraging in birds. Even though time can be objectively measured, it is a subjective sensation and is therefore subject to Weber-Fechner's law which states that the subjective intensity of a sensation (i.e., time elapsed) is proportional to the logarithm of the intensity of the stimulus causing it. Consequently, time estimation reflects Scalar Expectancy Theory, whereby the variation in time estimation error is proportional to the target criterion. The neural mechanisms underlying interval timing, and more specifically time estimation, have remained somewhat elusive in non-human species, especially birds. A putative candidate region for such temporal processing in birds is the associative endbrain area termed the nidopallium caudolaterale (NCL), which is linked to high-level cognition in birds and is considered the avian analogue of the mammalian prefrontal cortex. In the current experiment we recorded single-units in the NCL from two carrion crows trained on a delayed response task whereby arbitrary visual stimuli cued the subject how long to wait before making a response (1.5, 3, or 6 s). Timing behaviour from the crows reflect Scalar Expectancy Theory, such that variation in time estimations increased with the increased target estimation. During time estimation, we found many neurons that fire differentially for the three target estimations. Additionally, many neurons increased or decreased in firing across elapsed time in anticipation of making a response. Our results are the first to demonstrate how NCL neurons encode temporal information in birds, providing an important foundation for further investigation into the evolutionary constraints for time estimation in convergently-evolved intelligent vertebrate brains.

Disclosures: M. Johnston: None. **M.E. Kirschhock:** None. **A. Nieder:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.12

Topic: H.08. Learning and Memory

Support: University of San Diego, College of Arts and Sciences
TRiO McNair Scholars Program

Title: Examining the Role of the Endocannabinoid System in Elapsed Time Memory in Rats

Authors: V. CASTRO, E. FOLEY, A. ITO, J. M. WENZEL, *J. B. HALES;
Univ. of San Diego, San Diego, CA

Abstract: Time perception is a fundamental ability we use to judge duration of events, temporally organize our experiences, and decide when to initiate actions. Previous research suggests certain brain regions, such as the hippocampus, are critical for estimating elapsed time duration. The hippocampus expresses cannabinoid type-1 (CB1) receptors, and CB1 receptor activation via binding of endogenous or exogenous cannabinoids regulates neural signaling in these structures. In addition, cannabis acting at CB1 receptors 'speeds up' an organism's internal clock, meaning that humans and animals perceive time intervals as longer than they actually are after cannabis intake. However, it remains unclear how cannabinoids affect time discrimination processes in the hippocampus. To investigate this question, we trained rats on the Time Duration Discrimination (TDD) task, where they learned to discriminate between two durations to perform a correct learned response: turning left out of a delay box following a 10-second delay or right following a 20-second delay. After learning the discrimination, rats underwent surgery to implant guide cannulae bilaterally into dorsal hippocampus. Following recovery, rats continued daily testing on the task and, on select days, received intracranial infusions of the CB1/CB2 receptor agonist (WIN 55,212-2; WIN), the CB1 receptor antagonist/inverse agonist (Rimonabant), or a cocktail of GABAA+GABAB receptor agonists (Baclofen+Muscimol). Following drug testing, rats underwent satiety and LiCl devaluation procedures to test for habitual responding. Although none of the intra-hippocampal drug manipulations affected time discrimination in the well-learned TDD task, low dose Rimonabant slowed performance. Satiety and LiCl devaluation also had no effect on time discrimination, suggesting that behavior in the TDD task became habitual after extended training on the task. Despite there being no change in time discrimination performance, the LiCl devalued animals ate significantly less in the consumption test and took much longer to complete the TDD session, suggesting decreased motivation. Given that habitual behaviors are controlled by brain systems outside the hippocampus, such as the dorsal striatum, we suggest that intra-hippocampal manipulations did not affect timing behavior because the rats' performance on the well-learned TDD task had become habitual and no longer under control of the hippocampus.

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Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.13

Topic: H.08. Learning and Memory

Support: Secretaria de Educación Pública de México-Apoyo para el fortalecimiento de los Cuerpos Académicos

Consejo Nacional de Ciencia y Tecnología (CONACyT grants No. 701506 and INFR-281265)

Title: Cognitive evaluation with a timing task in a mouse model of chronic hydrocephalus induced by partial occlusion of the aqueduct of Sylvius

Authors: *T. CAMPOS ORDONEZ[1,2], M. EUDAVE-PATIÑO[2], J. BURITICÁ[2], O. GONZALEZ-PEREZ[1];
[1]Univ. of Colima, Colima, Mexico; [2]Lab. of Cognition and Comparative Learning, Univ. of Guadalajara-CEIC, Guadalajara, Jalisco, Mexico

Abstract: Hydrocephalus is a neurologic disturbance produced by the abnormal production, circulation, and absorption of cerebrospinal fluid (CSF), which expands the ventricular system. The aqueduct of Sylvius is the narrowest portion of the entire ventricular system that communicates the third ventricle with the fourth one. The occlusion of this structure or severe stenosis is considered as a predisposing factor for hydrocephalus development. Late-onset idiopathic aqueductal stenosis induces normal pressure hydrocephalus (NPH), motor and cognitive alterations in adults. To date, there is some information about the motor and cognitive skills of animal models replicating chronic NPH. Herein, we used an experimental model that induces chronic hydrocephalus in the adult mouse brain by producing a pre-aqueductual semiobstruction into the atrium of the aqueduct of Sylvius. After surgical procedure, we analyzed the hydrocephalus development on days 60 and 120 and sham-operated animals were used as controls. We included an additional group of hydrocephalus resolution in which we removed the obstruction and analyzed the morphological changes in the brain. All groups were tested in a peak procedure at a Skinner box. Four conditioning chambers (MED Associates, ENV-307A-D1) enclosed in sound-attenuating boxes were used. We found no differences in accuracy, precision or attention in peak, gap and distractors trials. Therefore, our model mimics cognitive-asymptomatic ventriculomegaly even 120 days of NPH, which still is considered a challenging clinical problem to be treated prophylactically or not. Thus, our mouse model may be feasible for studying the long-term cerebral alterations that occur during NPH or after its surgical resolution in the postnatal brain.

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Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.14

Topic: H.08. Learning and Memory

Support: National Natural Science Foundation of China

Title: Cognitive map facilitates storage of multiple sequences in working memory in the human brain

Authors: *Q. HUANG, M. WANG, H. LUO;
Sch. of Psychological and Cognitive Sci., Peking Univ., Beijing, China

Abstract: How the brain maintains a sequence of items in working memory (WM) via neural replay has been the main focus of previous studies, yet the storage mechanism for multiple sequences remains unknown. Here we examined whether and how the brain employs a cognitive map to retain two sequences in WM, by manipulating the consistency in sequential trajectory between two sequences within a common spatial map. Human subjects performed a two-sequence reproduction task wherein they needed to memorize both the spatial location and color of three serially presented targets and reported the three locations followed by three colors, in their correct order. Subjects' brain activities were recorded using electroencephalography (EEG). First, sequences with consistent spatial trajectory show better memory performance and less memory uncertainty than sequences with inconsistent trajectory. Most interestingly, during the location recalling phase when the color sequence is not currently task-relevant and would be recalled later, we revealed a forward, temporally-compressed reactivation profile for color sequence, and this neural replay further correlates with the color sequence memory performance. Taken together, human brains tend to deploy a cognitive map to facilitate the storage of multiple sequences, whereby a currently task-irrelevant sequence undergoes spontaneous neural reactivations to potentially consolidate its relationship to the underlying cognitive map.

Disclosures: Q. Huang: None. **M. Wang:** None. **H. Luo:** None.

Poster

660. Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 660.15

Topic: H.08. Learning and Memory

Support: JSPS Kakenhi #22H01101
JSPS Kakenhi #19H01771

Title: Non-spatial tactile perception: Is it affected by internal frame of reference?

Authors: *S. SUGIYAMA[1], Y. YOTSUMOTO[2];
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Abstract: Tactile information is represented within somatotopic maps, and the performance of tactile localization tasks depends on the body posture. Furthermore, tactile localization task performance depends on body posture. For example, temporal order judgment task (TOJ) performance, in which participants judge the order of stimuli applied to both hands, is significantly impaired when the arms are crossed. Although the tactile stimulus always

accompanies spatial information, one may perceive its temporal characteristics irrespective of the spatial location. In other words, the presence of somatotopy does not necessarily mean that spatial information *always* affects tactile perception.
While the temporal and spatial aspects of tactile perception are equally important, most tactile perception studies have examined its temporal aspects in the context of spatial localization. Hence, it remains unclear whether non-spatial tactile information is processed regardless of spatial location.
We examined whether the internal reference frame affects non-spatial tactile perception in the present study. We applied tactile stimulations using vibrating solenoids. Thirteen participants (4 females, average age 22.2 years) performed a frequency discrimination task with their arms crossed or uncrossed. The stimuli were presented either sequentially to each index finger (serial condition) or simultaneously to both index fingers (parallel condition). The results showed that discrimination performance was impaired when the arms were crossed. Moreover, impairment was more prominent in the parallel than in the serial condition.
If the non-spatial tactile processing is independent of the reference frames, crossing arms and creating a conflict between the reference frames would not have affected the performance. However, our results suggested otherwise: spatial information influenced tactile perception. Furthermore, the internal-external frame mismatch impaired non-spatial tactile perception.
We have concluded that tactile perception is susceptible to the internal frame even when perception is irrelevant to the localization of the stimuli and that spatial representation of the stimulus constrains somatosensory temporal processing.

Disclosures: S. Sugiyama: None. **Y. Yotsumoto:** None.

Poster

660. Timing and Temporal Processing

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 660.16

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH110594
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Army Research Office 78259-NS-MUR

Title: Surprise and recency in novelty detection in the primate brain

Authors: *K. ZHANG[1,2], E. BROMBERG-MARTIN[1], F. SOGUKPINAR[3], K. KOCHER[1], I. MONOSOV[1,2,3,4,5];
[1]Dept. of Neurosci., Washington Univ. In St. Louis, Saint Louis, MO; [2]Dept. of Biomed. Engin., [3]Dept. of Electrical and Systems Engin., [4]Dept. of Neurosurg., Washington University, St. Louis, St. Louis, MO; [5]Pain Ctr., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Humans and other primates learn by exploring novel objects, and neurons in many brain areas respond differently to novel versus familiar objects (Ranganath et al., 2003). However, the mechanism underlying neural novelty respond is not clear. Distinct theories propose that neuronal novelty responses reflect either computation of sensory surprise (Kumaran, 2007) or of stimulus recency (Vogels, 2015). However, these mechanisms could be interdependent. Novelty responses may arise from computations of both sensory surprise and recency, or neither. To study these issues, we recorded thousands of neurons across temporal cortex, amygdala, hippocampus, basal ganglia, and the prefrontal cortices while two monkeys participated in a behavioral procedure that tested the relationship of novelty, sensory surprise, and recency. During neural recording, monkeys viewed sequences of visual fractal objects. These fractals could be novel or familiar, more or less recent, and more or less surprising. A series of analyses of the neural population coding consistently showed that object novelty computation was intertwined with both sensory surprise and recency computations. Moreover, novelty neurons also showed anatomical organization, such that brain areas with a higher percentage of novelty responsive neurons tended to have higher percentages of sensory surprise responsive and recency responsive neurons. Control experiments indicated that these response patterns were not simply due to other aspects of the behavioral procedure such as general arousal or reward. Finally, we studied neuronal novelty-to-familiarity transformations during object learning across many days from repeated exposure to novel fractals. It is known that novel objects mediate behavior on different timescales, flexibly depending on context. But whether novelty-sensitive neurons display heterogenous timescales of novelty-to-familiarity learning to support such behavioral flexibility remains unclear. We found a wide spectrum of timescales in neurons' within-session learning and between-session forgetting within and across brain areas that are well suited to support flexible adaptive behavior to novel objects. Importantly, neurons that learned novel objects faster also forgot them faster, and neurons that learned more slowly also forgot them more slowly. And this could not be explained by session-by-session variation in learning. Our findings show that neuronal novelty detection arises on multiple timescales due to diverse but related computations of sensory surprise and recency, and shed light on the logic and computational underpinnings of novelty detection in the primate brain.

Disclosures: K. Zhang: None. **E. Bromberg-Martin:** None. **F. Sogukpinar:** None. **K. Kocher:** None. **I. Monosov:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.17

Topic: H.08. Learning and Memory

Support: NIH grant R01MH100121
NIH grant T32MH106454
NIH grant F32MH115585

Title: Linking Visual Predictability in Naturalistic Videos to Patterns of Event Segmentation in Development

Authors: *A. AMATUNI[1], A. DUTCHER[2], C. COUGHLIN[1], A. R. PRESTON[2];
[1]Psychology, [2]Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: The ability to organize continuous experience into discrete events develops through adolescence. Children identify different transitions between discrete events, i.e. event boundaries, relative to adults (Glebkin, Olenina, & Safronov, 2019), raising the question of how they organize experience into individual event memories. One prominent hypothesis suggests that neural systems which support event organization do so by monitoring changes in perceptual content, whereby moments of uncertainty mark boundaries within a continuous stream of experience (Reynolds, Zacks, & Braver, 2007; Zacks et al., 2007). Naturalistic video stimuli have been used to examine developmental differences in event segmentation. While rich stimuli offer ecologically valid substitutes to everyday experience, their complexity makes their contents difficult to analyze using traditional methods. It's unclear whether age-related improvements in segmentation relate to greater sensitivity to the inherent predictive properties of the input, as prior work using naturalistic stimuli has not objectively quantified perceptual prediction error or surprise. Here, we take preliminary steps to address this challenge. In this study, children (7-12 years old) and adults (N = 74) watched five naturalistic movies and were asked to press a button whenever they perceived an event boundary. We find that 7-9 year olds differ from adults in when they perceive boundaries ($p < 0.05$), suggesting they may interpret perceptual content differently. To relate these differences to the information in the stimuli, we quantify the inherent predictability of our videos using deep neural nets trained to predict the contents in the next frame given a preceding context. To solve this task, the networks learn predictive visual features of the stimuli, and their loss quantifies the difficulty in predicting the next frame at any given point in time. We link this predictive measure, along with extracted visual features, to participants' perceived event boundaries, offering a data-driven account of visual sensory surprise and perceptual similarity in event segmentation. We find that the points which older participants identify as boundaries are more consistently predictable, and that 7-9 year olds' choices correspond to less surprising perceptual transitions compared to adults and 10-12 year olds ($p < 0.01$). Furthermore, adults may choose boundaries at points when the perceptual similarity within an event is greater compared to segments defined by both 7-9 and 10-12 year olds ($p < 0.01$). Our results suggest a mature memory system may segment experience by maximizing within-event similarity.

Disclosures: **A. Amatuni:** None. **A. Dutcher:** None. **C. Coughlin:** None. **A.R. Preston:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.18

Topic: H.07. Long-Term Memory

Support: NRF 2021M3E5D2A010238
NRF 2021M3A9E4080780

Title: Valence re-mapping processes for contextual association of mixed emotions to spatial scenes

Authors: *H. S. YANG, S. LEE;
Brain and Cognitive Sci., Seoul Natl. Univ., Gwanak-gu, Korea, Republic of

Abstract: Experiencing an emotional event in an environment affects one's memory representation of that context. However, most studies thus far have investigated the association of a single emotion to a single context, despite the fact that realistic situations often involve opposing emotional experiences in the same context. Regions such as the vmPFC, amygdala, and hippocampus have been implicated in the retrieval of both positive and negative emotions, but their roles in associating mixed emotions to a context remain unknown. In this study, we used fMRI to test whether a shift in neural activity occurs during mixed-valence contextual memory, specifically whether mixed emotion associations would additionally activate processes for value-updating in brain regions such as the OFC and dLPFC, compared to remembering single emotion associations. 44 participants (18 male, 26 female) were scanned while viewing neutral spatial scenes, each of which were then presented with two successive emotional foreground images overlaid on top. Each of the two images were either positive (P) or negative (N), and participants were asked to imagine what happened in the scene and to provide valence ratings while viewing each of them. After repeating this process ten times, only background scenes (without the foreground images) were presented, and participants were asked to rate the valence associated with each scene. After completing 8 runs, participants completed a scene recognition task outside of the scanner, using only background scenes that appeared previously. During the scene-only phase, participants rated the single-emotion conditions (NN & PP) as strongly negative or positive, respectively, whereas average ratings of the mixed-emotion conditions (NP & PN) were closer to zero and showed higher individual variability. Moreover, scenes from the single-emotion conditions were recognized more accurately than those from the mixed condition (84%, 78%, respectively). fMRI results showed that single-emotion conditions generally elicited high Hp activation and reversed vmPFC-amygdala activation for positive and negative emotions (i.e., higher vmPFC for positive, higher amygdala for negative). Mixed-emotion conditions, however, resulted in lower hippocampal activation and low activation in both the vmPFC and amygdala. The OFC and vlPFC, on the other hand, were highly activated, suggesting an updating or remapping of two different context-valence associations. These findings shed light on the role of arbitration-like processes in our memory of emotional experiences.

Disclosures: H.S. Yang: None. **S. Lee:** None.

Poster

660. Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 660.19

Topic: H.07. Long-Term Memory

Support: NRF-2021M3E5D2A01023891
NRF-2020K1A3A1A19088932

Title: Representations of semantic, spatial, and temporal distance in episodic memory retrieval are mediated by slow and fast theta oscillations

Authors: *J. LEE[1], S.-E. PARK[2], S. A. LEE[1];
[1]Seoul Natl. Univ., Seoul Natl. Univ., Gwanak-gu, Korea, Republic of; [2]Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Episodic memories can be defined by *what* happened, *where* it happened, and *when* it happened. Studies have shown that the different components of memory can be dissociated by their neural correlates and their heterogenous impairment in memory disorders. However, recent studies also raise the possibility that common domain-general mechanisms of cognitive mapping may be involved in the coding of semantic distance (*what*), spatial distance (*where*), and temporal distance (*when*). Given these findings, we hypothesized the simultaneous existence of both shared (domain-general) and unique (domain-specific) neural correlates across the *what*, *where*, and *when* components of episodic memory. To see such potential memory representations at work during memory retrieval, we tested 47 college students (21 female, age range 20-27) on a scene-based episodic memory task designed to assess the components separately. After showing them a series of 10 scenes, the *what* retrieval test required a choice between two objects the one that was missing from a particular scene; *where* retrieval required a choice between two scenes in which the spatial location of an object differed; and *when* retrieval required participants to choose which of two scenes came first during encoding. To measure neural activity during the memory retrieval phase (response time 2.3 sec on average), we recorded scalp EEG (32-channels) and calculated spectral power using Morlet wavelet transformation.We tested for correlations between cognitive distance (semantic, spatial, and temporal) and slow (2.5-5Hz) and fast (5-8.5Hz) theta power. First, during the first half of the retrieval phase (over the first second after stimulus onset) we found a unique neural signature of time - a negative correlation of slow theta power with temporal distance. In the second half of the retrieval period, we found that slow theta power was correlated with all three types of distance, while fast theta power was correlated with spatial and temporal distances. We take these results to be indicative of: 1) a domain-general, abstract representation of distance for cognitive mapping, 2) a spatiotemporal, perhaps navigational distance correlate in fast theta, and 3) an immediate (early) modulation of slow theta in temporal order memory retrieval. Although the first two markers replicate past studies using scalp EEG and iEEG, the third marker of temporal distance is novel and may signify a unique neuro-cognitive process required in temporal organization of memory.

Disclosures: J. Lee: None. **S. Park:** None. **S.A. Lee:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.20

Topic: H.07. Long-Term Memory

Support: NRF-2021M3E5D2A01023891
NRF-2019R1F1A1062801

Title: Temporal binding of "where" as an indicator of episodic memory network engagement

Authors: *J. SHIN[1,2], S. LEE[2];
[1]KAIST, Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [2]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Episodic memory (EM) is a key cognitive function that enables us to remember the details of past experiences including objects, locations, and temporal order (*what, where,* and *when*). While humans can flexibly associate EM components into a single event memory, a detailed neural account of how *what* and *where* information are bound together in time is still unclear. In this study, we tested whether remembering a temporal sequence of *where* is dissociable from remembering *what* information in EM.
Subjects (n=33, 11 female) performed an EM task in a virtual environment in the fMRI scanner. They were asked to remember an event in which objects (*what*) moved one by one into an array of boxes (*where*) in a specific order (*temporal binding*). After an interference task, they were asked to recall the entire event in their mind while looking at the still scene of the room (retrieval), and then asked to respond by using a controller to reenact the entire sequence of objects and locations. Subjects performed at ceiling in choosing the correct objects and locations (*what* and *where*) but made errors in binding them into a temporal sequence (*what-when* and *where-when*). Interestingly, error patterns for what-when and where-when varied across individuals, and the subjects who showed preserved where-when binding even when they made errors tended to have higher full EM accuracy (*what-where-when*).
fMRI results showed that the retrieval of *what-where-when* associated event induced activation of the visual episodic memory network including MTL, OFC, precuneus, and fusiform gyrus. The EM trials with perfect memory showed higher EM network activation than the error trials, especially in the posterior part of the hippocampus (HPC) and entorhinal cortex. Next, the error trials with higher *where-when* preservation (i.e., better location sequence accuracy and worse object sequence accuracy) induced more activation in the right anterior HPC (particularly in the subiculum, $t=2.69$, $p=0.007$) as well as fusiform gyrus. *Where-when* binding was also associated with higher functional connectivity of the anterior HPC with the temporal and parahippocampal cortex. Moreover, individual differences were also reflected in the activation of the EM network. Subjects with higher *where-when* utilization showed higher activity in OFC and fusiform gyrus, even when accounting for their generally higher EM accuracy. Our study provides insight into how the temporal binding of different EM components is represented in the brain and suggests that spatiotemporal (*where-when*) binding is indicative of an active EM network and predictive of successful episodic memory.

Disclosures: J. Shin: None. **S. Lee:** None.

Poster

660. Timing and Temporal Processing

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Topic: H.07. Long-Term Memory

Support: NRF 2021M3E5D2A01023891
NRF 2020K1A3A1A19088932

Title: Distinct neural mechanisms underlying individual differences in the retrieval of temporal information in episodic memory

Authors: *S.-E. PARK, J. LEE, S. LEE;
Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Despite many previous studies that showed dissociable neural correlates of the *what, where, and when* components of episodic memory, there is still a lack of a domain-based explanation of individual differences in memory performance. Given the particularly fragile nature of temporal information (i.e. *when* in comparison with *what* and *where)* in Alzheimer's disease, finding specific neural markers of individual differences in temporal memory may be useful for both scientific and clinical purposes. We investigated EEG activity during the retrieval of episodic memory in 47 college students using a scene-based task designed to isolate *what*, *where*, and *when*. Subjects were asked to remember 10 scenes during the encoding without being informed about the condition (i.e. *what, where and when*). *What* retrieval required subjects to choose the correct object that was in a particular scene; *where* retrieval required them to choose the correct spatial location of an object from two possible scenes; and *when* retrieval require them to choose the scene that they saw first. We extracted the spectral EEG features by applying wavelet transform. First, we observed a significant correlation between *what* and *where* but not with *when*, suggesting that *when* retrieval may be functionally independent from the other two components. Based on the behavioral measures, we divided subjects into three groups using k-means clustering: The first two groups (Group G - good overall performance and B - bad performance) showed similar accuracy profiles across the three components. The third group performed well specifically in the *when* condition compared to the other components (Group GT - good temporal memory only). To test whether these "cognotypes" in behavior can be explained by their neural activities, we first compared the theta power (a well-known neural marker of memory) across the three groups. Subjects who performed well at *what* and *where* (Group G) showed higher theta power than the other groups during the retrieval (16 out of 32 channels for $p<0.05$), but this pattern was not observed for *when* task. Instead, performance on the *when* retrieval depended on how well the theta power was modulated across temporal distance between two scenes. Group G and TG (high performers for *when*) showed modulation of their theta power for coding temporal distance (correlation against zero: $p<0.001$) while Group B did not show any neural pattern associated with temporal distance. In summary, we found a distinct behavioral pattern and its corresponding neural markers for temporal memory retrieval which can be potentially useful for explaining and predicting individual differences in vulnerability to episodic memory disorders.

Disclosures: S. Park: None. **J. Lee:** None. **S. Lee:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.22

Topic: H.07. Long-Term Memory

Support: IITP Grant 2019-0-01371-003
NRF 2021M3E5D2A01023891
SYedu

Title: Neural correlates of boundary-based episodic memory organization

Authors: *Y. RAH[1], J. SHIN[2,1], S. LEE[1];
[1]Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Program of Brain and Cognitive Engin., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Spatial boundaries such as doorways and walls provide strong contextual cues by which continuous experience is partitioned into discrete chunks. In order to investigate the extent to which boundary-processing in regions such as the hippocampal formation, PPA, and OPA is associated with the utilization of spatial boundaries for episodic memory organization, we compared episodic memory (what-where-when binding) performance between virtual rooms with and without a boundary in its center. While in the fMRI scanner, participants (n = 35, 17 female, mean age = 24.34) were instructed to watch a 30-sec-long sequence of five objects moving one-by-one into five different baskets in a virtual room. Half of the virtual environments contained a low, freestanding wall in the middle of the basket array. After completing an interference task, participants were then asked to mentally recall the event while viewing a still image of the scene for 10 seconds, and then finally asked to enter their response using a controller to move objects to their respective locations. Although there was no difference in the group averages in memory performance between the boundary condition and no-boundary condition, given the wide range of individual differences, the participants were divided into two groups based on whether the boundary was beneficial for their memory or not. Those who benefitted from the presence of the boundary (n=16) showed higher activation of the posterior hippocampus during the retrieval period in the boundary condition (compared to the no-boundary condition), and this enhancement of sequential episodic memory binding was not simply attributable to an increase in spatial memory accuracy. Interestingly, boundary-users showed different memory performance across portions of the event sequence that traversed the boundary from those that did not (across-boundary - within-boundary memory accuracy); and this difference was also correlated with the activation of the hippocampus and entorhinal cortex (only in the boundary-users). Moreover, we found that the scene network (e.g., OPA and PPA) was also more highly activated during encoding for the boundary users and was associated with subsequent memory in the boundary condition during retrieval. These results suggest that boundary-like structures in the environment help organize events into sequential episodes, and

that this process is subserved by the hippocampal formation and boundary- perception processes in the cortex.

Disclosures: Y. Rah: None. **J. Shin:** None. **S. Lee:** None.

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.23

Topic: H.08. Learning and Memory

Support: ANR JCJC
NARSAD
EU Horizon 2020; Marie Curie 754387

Title: A hippocampal-thalamic-cortical network modulates remote memories.

Authors: *C. ANDREWS[1], F. ÁVILA-GÁMIZ[2], N. BALCELLS PICAZA[1], G. VETERE[1]; [1]Brain Plasticity Unit, ESPCI, Paris, France; [2]Psicobiología y Metodología de las Ciencias del Comportamiento, Univ. de Málaga, Málaga, Spain

Abstract: When a memory is kept for the long-term, the gist is stored but the context specific information is lost. We can study memory processes in Pavlovian fear conditioning, where mice learn to associate a context with an aversive experience. A spatial representation network is active to create a cognitive map of the contextual information during memory acquisition. Over time the detailed contextual information is lost. Brain-wide mapping of immediate early gene revealed a strong hippocampal-thalamic-cortical signature in the brain network active during remote memory recall. We aim to unravel the contribution of hippocampal-thalamic-cortical circuitry in this process. Graph theory analysis of memory networks showed that the anterodorsal thalamic nucleus (ADn) activity positively correlates with other regions during recent memory recall but, during remote recall, this pattern switched and ADn activity negatively correlated with other regions. This suggests that the inhibition of the ADn by other regions is required during the recall of a remote memory. Using optogenetics in vivo, we targeted the anterior cingulate cortex (aCC)-ADn pathway and the hippocampal CA3-ADn pathway, as these regions are important in long-term memory recall. Results show that optogenetic inhibition of CA3 excitatory projections to the ADn, leads to impaired memory performances at a remote time point. We also gathered preliminary results showing an aCC-ADn regulation of recent memory traces. All together these data suggest a time depend engagement of the hippocampal-thalamic-cortical network in regulating long lasting emotional memories with a spatial component.

Disclosures: C. Andrews: A. Employment/Salary (full or part-time):; Cerebral codes and circuits connectivity, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, Paris, France. **F. Ávila-Gámiz:** A. Employment/Salary (full or part-time):; Departamento de Psicobiología y Metodología de las Ciencias del Comportamiento, Instituto de Investigación

Biomédica de Málaga (IBIMA), Facultad de Psicología, Universidad de Málaga, Spain, Cerebral codes and circuits connectivity, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, Paris, France. **N. Balcells Picaza:** A. Employment/Salary (full or part-time):; Cerebral codes and circuits connectivity, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, Paris, France. **G. Vetere:** A. Employment/Salary (full or part-time):; Cerebral Codes and Circuits Connectivity team, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, 10 rue Vauquelin, 75005 Paris, France..

Poster

660. Timing and Temporal Processing

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Program #/Poster #: 660.24

Topic: H.08. Learning and Memory

Support: Marie Curie Individual Fellowship Project Number 896825
ANR JCJC
NARSAD

Title: Laterodorsal Nucleus of the Thalamus neuronal contribution to long-term contextual fear memory consolidation

Authors: *J. P. CASANOVA, C. POUGET, G. VETERE;
ESPCI, Paris, Paris, France

Abstract: The Laterodorsal Nucleus of the Thalamus (LDn) is part of the head direction cell system. Recently, it has been shown to be involved in memory consolidation. However, little is known about its function either as part of the head direction cell system or regarding its activity during the memory process. Specifically, we wanted to study how emotion affects head direction cell tuning during memory acquisition, consolidation, recall and generalization. To this aim, we recorded the activity of hundreds of cells from the LDn using *in vivo* Calcium imaging with miniaturized fluorescence microscopes, in mice subjected to a contextual fear memory protocol. Briefly, mice were trained to associate a context to an aversive stimulus (foot shocks), and then re exposed to this same context (context A) and a neutral one (context B), at recent (1 day and 2 days after training, respectively), and at remote (21 and 22 days after training) time point. Freezing was used as a behavioral measure of memory index. Thus, we found around 30% of LDn neurons whose activity is tuned to head orientation (head direction cells), confirming a previous report. We then tracked the same cells over time to visualize their different involvement at a remote time point. We finally analyzed different cortical projections to the LDn and their role in memory consolidation processes.

Disclosures: J.P. Casanova: None. **C. Pouget:** None. **G. Vetere:** None.

Poster

660. Timing and Temporal Processing

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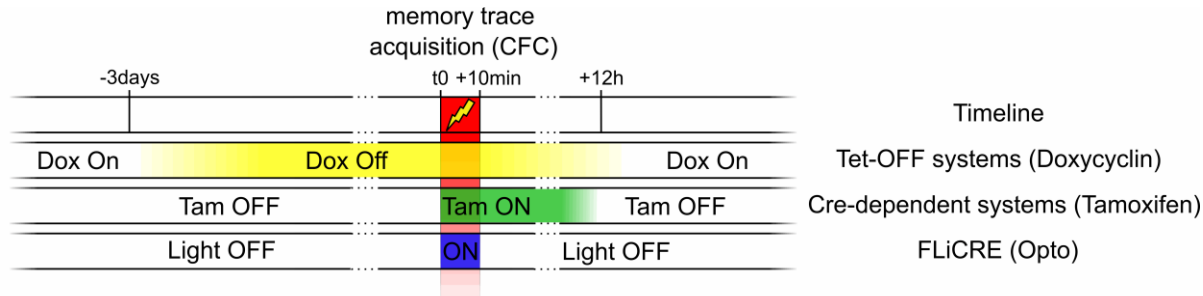
Program #/Poster #: 660.25

Topic: H.08. Learning and Memory

Title: A novel approach for specifically tagging and reactivating engram cells: a next step towards precise engram manipulation?

Authors: *C. POUGET[1], G. VETERE[2];
[1]Brain Plasticity Unit, ESPCI Paris, Paris, France; [2]Brain Plasticity Unit, ESPCI CNRS, Paris, France

Abstract: Engrams, physical substrates of memory traces, are defined as subsets of neurons that: are active during the acquisition of a memory trace, that undergo synaptic and cellular changes, and that are reactivated when said memory trace is recalled.
It has been shown that artificially reactivating even only part of an engram is sufficient to recall the associated memory trace, or that silencing it results in an impairment of memory recall. While displaying groundbreaking results in the engram study field, these studies use a combination of immediate early gene (IEG) promoters and Cre/Tet-ON controlled by intake of medicine (Doxycyclin, Tamoxifen) via food/water to tag engram cells during memory acquisition. Due to slow kinetics of IEGs and the imprecision of the administration method, the tagging time window remains open for a longer time than wanted.
To properly display the true mechanisms of memory engrams by reaching a higher level of time specificity, we decided to test FLiCRE (Kim et al. 2020), a novel light and calcium-gated molecular labeler of active neurons. Shining blue light at active FLiCRE-infected neurons allows the transcription of any protein (e.g an opsin) with second precision, making it the perfect tool for engram cell investigation.
Our goal was to tag cells in mice dentate gyrus (DG) during contextual fear conditioning, and reactivate those specific engram cells in a novel neutral environment (an open field) to elicit an artificial fear response (measured with freezing) in it.
To do so, we used FLiCRE to induce expression of a red-shifted excitatory opsin (bReaChES) in tagged cells. Both male and female mice were used. Animals that received the actual treatment showed significantly more freezing during test in light ON compared to light OFF periods, while control animals did not.



Disclosures: **C. Pouget:** None. **G. Vetere:** None.

Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 661.01

Topic: H.09. Spatial Navigation

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Title: Flexible cue anchoring strategies enable stable head direction coding in both sighted and blind animals

Authors: *K. ASUMBISA, A. PEYRACHE, S. TRENHOLM;
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Abstract: Vision plays a crucial role in instructing the brain's spatial navigation systems. In the absence of vision, the remaining sensory systems attempt to fill in the previously dominated role of vision. However, how the brain's navigational system is affected, as well as the strategies it adapts to facilitate spatial awareness following vision loss remains an open question. To explore this, we implanted 32-channel silicon probes to record from HD cells in the anterodorsal thalamus (ADn) of freely behaving sighted and blind animals. For blind animals, the recordings were done in congenitally blind (Gnat1/2^{mut}) and later onset blind mice (rd1). First, we found that a vast majority of cells in ADn of blind animals were modulated by HD and exhibited stable and robust HD tuning. In contrast, placing sighted animals in darkness impaired their HD cell tuning. The timing of vision loss appeared to affect the stability of HD cell tuning, with congenitally blind mice exhibiting less refined tuning compared to late-onset blind mice. Additionally, we observed that HD cells in blind animals were primarily anchored to floor olfactory cues, showing concomitant shifts in their preferred firing directions following floor rotations. By ablating olfactory sensory neurons (OSNs) via intranasal administration of zinc sulfate, HD tuning became unstable in blind animals. To further explore network activity following loss of stable tuning, we projected the population activity of HD cells unto a low-dimensional manifold using Isomap, revealing that a 1D ring manifold persisted in the absence of stable HD tuning. In conclusion, we show that blind animals have stable and robust HD tuning, meaning that the HD system is flexible in which sensory system it can use for obtaining reliable cue anchoring: sighted animals predominantly use visual signals, whereas blind animals use olfactory signals. Finally, in the absence of both visual and olfactory cues, the ring attractor in ADn remains intact, but becomes decoupled from external cues.

Disclosures: **K. Asumbisa:** None. **A. Peyrache:** None. **S. Trenholm:** None.

Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 661.02

Topic: H.09. Spatial Navigation

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Title: Traveling UP states in the Post-Subiculum Reveal an Anatomical Gradient of Intrinsic Properties'

Authors: *D. MEHROTRA[1,2], A. J. DUSZKIEWICZ[2,3], D. LEVENSTEIN[2,4], A. PEYRACHE[2];
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Abstract: Functional gradients along an anatomical axis are a common feature of the spatial navigation system. Along the dorsoventral (DV) axis of the medial entorhinal cortex (MEC), grid cells increase in grid spacing and head-direction (HD) cells decrease in their tuning specificity, accompanied by a decrease in rectifying currents. These observations beg the question of whether such functional gradients are intrinsic to the MEC or are inherited from the head-direction cortex (HDC i.e., the post-subiculum), the main cortical recipient of the thalamic HD signal, and is critical for the generation of the grid code. To investigate the possibility of a functional gradient in the HDC, we used 64-channel linear electrode arrays to conduct simultaneous population recordings along the DV axis of the HDC in adult male mice. In contrast to MEC, where HD cell tuning shows a gradual increase along the DV axis, HD tuning in HDC was uniform across the DV axis. However, we found that a gradient of spiking properties emerged during non-Rapid Eye Movement (NREM) sleep. NREM sleep is characterized by aperiodic alternations of neuronal activity between “UP” states, during which neurons are depolarized, and “DOWN” states, during which most cells are hyperpolarized, and large slow waves are seen in the local field potential. We found that UP states began earlier in the dorsal HDC than in the ventral part, resulting in sequential activation of HDC cells along the DV axis that travelled with a speed of ~5mm/s. In contrast, UP-to-DOWN transitions were near-synchronous along the DV axis. As a result, slow waves were synchronous along the DV axis while post-slow wave gamma-band activity mirrored the DV travel of spiking. To understand the mechanism underlying this gradient, we built a computational model with a linear array of recurrently connected units and compared the spatiotemporal properties of DOWN states generated by various biophysical gradients. The model uniquely matched experimental observations with a gradient in the strength of a rectifying current, suggesting that the sleep-

related gradient in HDC has a common anatomical origin to the functional gradient in MEC. In the model, sequential activation results from stronger rectifying currents in dorsal cells, which brings them back to resume activity which then propagates via lateral connections. Further experiments will investigate the cellular basis of this gradient and explore the anatomical and functional relationship between the HDC and the MEC to provide further insight into the organization of the limbic system and its role in spatial navigation and memory.

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Poster

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Title: A predictive learning model for autonomously generated replay in the hippocampus

Authors: *D. LEVENSTEIN[1], A. PEYRACHE[2], B. A. RICHARDS[3];
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Abstract: During waking behavior, the hippocampus is thought to represent an animal's location in a cognitive map of the environment. During periods of sleep and immobility, it uses that map to autonomously simulate behavioral trajectories in previously-explored environments. These "replay" events are thought to support the formation of long term memories, integration of information from past experiences into overarching world models, and even planning for future behaviors. Intriguingly, replay can range from veridical replay of actual experiences to diffusive motion and generative trajectories never taken by the animal. The current best model of these capabilities is a multi-chart continuous attractor network (CANN), thought to be supported by recurrent connections in CA3. However, CANNs require hand-wired connections or a preexisting correspondence between cells and environmental locations, and it's unclear how environment-specific CANNs can be learned from sensory data alone. Aligning with classical predictive theories of hippocampal function, recent work has shown that predictive learning of future sensory observations can result in cells with spatial tuning similar to hippocampal cells. However, it's unknown if this tuning reflects a continuous attractor that can be used to simulate unexplored trajectories.
To study the generation of replay in predictive networks, we simulated a recurrent neural

network which learns to predict sensory observations in a gridworld environment. We found that predictive learning alone is insufficient to develop a continuous attractor or autonomously generate trajectories offline. While the network did develop a linearly-decodable spatial representation with tuning curves akin to hippocampal place cells, it relied on external inputs and was unable to maintain an internal representation during a sleep-like state. However, we found that a predictive learning architecture inspired by the hippocampal theta oscillation, in which recurrent connections are used to project multiple timesteps into the future, was able to replicate both the spatial representation and offline simulation abilities of the hippocampus. In this theta-predictive RNN, locations decoded during offline activity showed spatiotemporal continuity akin to activity in the environment. We then used this network to study how various hippocampal-inspired features shape the properties of offline replay in the network. Together, these results support the role of online prediction for the production of offline simulation, and open the door for future work on the mapping and replay abilities of the hippocampus and the computations they support.

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Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

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Support: CIHR Project Grant 155957
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Title: Medial entorhinal cortex and thalamic HD cells are coordinated during sleep

Authors: *G. R. VITE, R. LU, Q. DING, S. ANGELES-DURAN, L. LI, A. PEYRACHE; Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Flexible navigation requires signals that are updated irrespective of the external conditions. The demonstration that pairwise coordination of both grid and head-direction (HD) cells is maintained during sleep when external inputs are largely reduced, has provided experimental evidence for attractor dynamics in these systems, a property ensuring reliable population coding at all times. Specifically, this was shown separately for the HD cells of the anterodorsal nucleus (ADN) of the thalamus and for HD and grid cells of the medial entorhinal cortex (MEC). Inactivation studies previously showed that ADN HD cells are necessary for normal grid cell activity, begging the question of the exact role of the thalamic HD cells in MEC population activity. To address this problem, we performed simultaneous electrophysiological recordings of thalamic HD cells and the MEC during wake and sleep (n=3). Our results show that the coordination between HD cell pairs in ADn and MEC is preserved after environmental changes, during a cue rotation experiment; and during all phases of sleep, non-Rapid Eye

Movement (NREM) and REM, where the angular offset of the preferred direction during wake predicts the correlation during sleep. Finally, we found that the cell pairs correlation in ADn is more constant along the time of the recording in comparison with MEC, which suggests that the attractor present in ADn is more rigid. Our results show that neuronal population activity is internally organized, i.e. across all states, in distributed networks of the spatial navigation system, not only in local networks. References: 1. Peyrache et al. (2015) Nat. Neurosci. 18, 569-575. 2. Gardner et al. (2019) Nat. Neurosci. 22, 598-608. 3. Winter et al. (2015) Science. 22, 870-874.

Disclosures: G.R. Vite: None. **R. Lu:** None. **Q. Ding:** None. **S. Angeles-Duran:** None. **L. Li:** None. **A. Peyrache:** None.

Poster

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Title: Which geometric coding scheme drives neurons in postrhinal cortex - the environmental centroid or local boundaries?

Authors: *P. A. LACHANCE, J. S. TAUBE;
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Abstract: Animals use the geometry of their local environments to orient themselves during navigation and spatial behavior. This geometric information arrives in the brain from the animal's first-person perspective, and therefore is considered egocentric or self-centered. Single neurons have recently been recorded in the rat postrhinal cortex (POR) that appear to encode geometric elements of the environment in an egocentric reference frame, such that they fire in response to the egocentric bearing and/or distance of the environment center or boundaries (LaChance et al., 2019; Gofman et al., 2019). However, experiments conducted with these neurons have been restricted to highly symmetrical environments with opaque walls (such as squares and cylinders), so the specific geometric underpinnings (i.e., coordinate system) of this egocentric signal remain largely unexplored. One major question is whether these neurons truly encode high-level global parameters, such as the bearing/distance of the environment centroid, or if they are simply responsive to the bearings and distances of nearby walls. To address this issue, we recorded from POR neurons as rats foraged in environments with different geometric layouts, and modeled their responses based on either global geometry (centroid) or local boundary encoding. Instead of favoring one coding scheme over another, we found that POR neurons largely split into two groups: either centroid-encoding or local-boundary-encoding cells, with

each group lying at one end of a continuum. We also found that distance-tuned cells tend to scale their linear tuning slopes in a scaled-down small environment, such that they lie somewhere between absolute and relative distance encoding. Finally, we found that POR cells largely maintain their bearing preferences, but not their distance preferences, when exposed to environments that contain different boundary types (opaque, transparent, drop-edge), suggesting that there are different inputs that drive the bearing and distance signals. Overall, we conclude that the egocentric spatial correlates encoded by POR neurons comprise a largely robust and comprehensive representation of environmental geometry.

Disclosures: P.A. LaChance: None. **J.S. Taube:** None.

Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

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Topic: H.09. Spatial Navigation

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Title: Mesoscale calcium dynamics observed across the cortex in freely moving mice identify brain states during spatial navigation and learning

Authors: *M. RYNES[1], D. SURINACH[2], K. SAXENA[2], E. KO[1], A. D. REDISH[3], S. B. KODANDARAMAIAH[2];
[1]Biomed. Engin., [2]Mechanical Engin., [3]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Successful goal-directed navigation requires an integration of multiple streams of sensory environments, incorporation of those representations into goal-related plans, and actuation of those goal-related plans. How this multi-sensory information is processed across the cortex during navigation is not well understood. To investigate this, we recorded cortex-wide calcium dynamics in Thy1-GCamp6f mice (n = 11) using the mini-mScope (Rynes*, Surinach* et al 2021), a head-mounted miniaturized microscope capable of mesoscale calcium imaging of large swathes of the dorsal cortex, in mice solving the Barnes maze, an established spatial learning task. The Barnes maze consists of a single escape hole in the presence of mildly aversive sensory stimuli (bright light). Mice learned to successfully navigate to the escape hole predominantly using a random search strategy in early trials, progressing to serial search and then spatial search strategies in later trials. Latency to first reach the goal progressively decreased as learning progressed, with a mean latency of 51 seconds on the first day decreasing to 14 seconds in late trials. K-means clustering revealed spatially conserved patterning of calcium activity clusters across trials for each mouse, with 18-24 clearly defined clusters of pixels per hemisphere emerging. We further observed cortex-wide calcium activity to be discretized into distinct epochs. Using an unsupervised Greedy state search algorithm, we

clustered cortical activity into sets of neural activity states, with K = 6-9 states emerging in each mouse. Finally, convolution neural networks
trained on calcium imaging data could decode the spatial location of mice with an accuracy of 76% in the outer sectors of the maze and 62% in the inner sectors of the maze. A holistic view of the activity across the cortex during a rapid, ethologically relevant spatial learning task revealed neural states indicative of distinct sets of brain-wide circuits being recruited during navigation behaviors.

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Poster

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Title: Diverse long-range projections convey position information to the retrosplenial cortex

Authors: *A. GARVERT, M. GIANATTI, K. VERVAEKE;
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Abstract: The hippocampus supports spatial navigation and episodic memory by routing position information to downstream brain areas. However, it remains unclear whether downstream areas also share position information with each other. Answering the question of how circuits share positional information requires measuring the activity of thin afferent axons in a projection specific manner. Because this is technically challenging, no comprehensive mapping of long-range inputs to a position modulated circuit has yet been done. Here, we addressed this question by studying the mouse retrosplenial cortex (RSC), a major output structure of the hippocampus that has position modulated neurons. First, we determined the main input pathways to RSC using retrograde viral tracing methods. This identified the orbitofrontal cortex, the secondary motor cortex, anterior cingulate cortex, posterior parietal cortex, primary and secondary visual cortex and various thalamic nuclei as the major projections to RSC. Next, we functionally characterized these input pathways, performing subcellular-resolution two-photon microscopy in head-fixed mice that perform a foraging task on a linear track in darkness. We selectively expressed GCaMP6s in each of the identified presynaptic areas and measured calcium signals of their axons in the agranular RSC. Surprisingly, most projections convey position information, but with key differences. Using Bayesian decoding, we found that axons from the secondary motor cortex transmit by far the most position information. Axons from the posterior

parietal- anterior cingulate- and orbitofrontal cortex and thalamus also convey position information but substantially less. Axons from the primary- and secondary visual cortex have a negligible contribution. This demonstrates that circuits downstream of the hippocampus share position information in a projection-specific manner.

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Poster

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Title: A cell type specific error correction signal in mouse posterior parietal cortex

Authors: *J. GREEN[1], C. BRUNO[1], S. HRVATIN[2], D. E. WILSON[1], M. E. GREENBERG[1], C. D. HARVEY[1];
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Abstract: Neurons in posterior parietal cortex contribute to the execution of goal-directed navigation and other decision-making tasks. Although molecular studies have catalogued over fifty cortical cell types, it remains unknown whether these cell types display distinct activity and connectivity patterns in this region and what distinct functions they serve during goal-directed navigation. Here we identify a subset of somatostatin (Sst) inhibitory neurons in mouse posterior parietal cortex that excite one another and synchronously activate during navigational course corrections. We obtained repeatable experimental access to these cells using an adeno-associated virus in which gene expression is driven by an enhancer that functions specifically in a subset of *Calb2+* and *Hpse+* Sst cells. We found that during goal-directed navigation in a virtual environment, this subset of Sst neurons activates in a synchronous pattern that is distinct from the activity of other surrounding neurons, including other Sst neurons. Photostimulating this subset of Sst neurons increases activity in nearby cells of the same subtype, but not other Sst neurons, revealing a self-excitation circuit motif that likely contributes to the synchronous activity of this subset of Sst neurons. Remarkably, these cells are selectively activated as mice execute course corrections for deviations in their virtual heading during navigation toward a reward location, both for self- and experimentally induced deviations. We propose that this

subtype of Sst neurons provides a self-reinforcing and cell type-specific error-correction signal in posterior parietal cortex that may aid the execution or learning of accurate goal-directed navigation trajectories.

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Poster

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Title: Investigating the emergence of neural circuits for navigation in developing rats using wireless technology.

Authors: *T. O'DRISCOLL, L. MUESSIG, T. WILLS;
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Abstract: The neural representation of space is encoded by spatially-modulated neurons including head direction cells (HD cells), place cells, and grid cells. Previous work has shown that these cell types emerge sequentially in rat postnatal development (Wills, 2010; Langston et al., 2010).
Typically, spatial cognition experiments using in vivo electrophysiology are made as a single animal forages in an open-field environment while tethered to an acquisition system. In developmental studies, this requires the removal of the rat pup from its homecage, mother and littermates. Wireless technology is emerging as a promising alternative: neural data loggers permit the recording of single-unit neuronal activity in an animal's homecage, thereby tracking spatial cell development while minimising disruption of early sensory experiences. The first aim of this study was therefore a proof-of-concept that wireless recordings of spatial cells in rat pups are comparable to standard techniques.
The second aim was to investigate whether the maturation of HD cells follows a different trajectory in a naturalistic environment to that previously observed in traditional open-field recordings (Tan et al., 2015; Bassett et al., 2018). To address this, ensembles of HD cells were wirelessly recorded in the homecage from P12 to P16. Preliminary results suggest that HD cells are more stable in the open field than in a naturalistic environment (homecage). By studying the ontogeny of these cells, we hope to better understand the neural basis of spatial learning and memory.

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Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

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Topic: H.09. Spatial Navigation

Support: TKP20214

Title: Feedback inhibition in the entorhinal cortex mediated by neurogliaform cells

Authors: *S. SZOCS, N. HENN-MIKE, A. AGOCS-LABODA, C. VARGA;
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Abstract: The role of local GABAergic inhibitory neurons in generating the entorhinal specific cell activities is still not entirely known. Several studies focused on the function of parvalbumin+ fast spiker interneurons, and only limited data has been published on the connectivity-matrix of other GABAergic cell types. Several interneurons are localized in the layer I, where apical dendrites of layer II-V pyramidal and stellate cells are located. The majority of these critically positioned interneurons are neurogliaform cells. Neurogliaform cells have been shown to elicit prolonged GABAA and GABAB receptor mediated inhibition in the neocortex and hippocampus in virtually all cell types which are located within the range of the rich axonal clouds of the neurogliaform cells. They are generally supposed to perform feed-forward inhibition: in the somatosensory cortex thalamic input; in the dentate gyrus entorhinal input; in the CA1 entorhinal and CA3 inputs give excitatory synapses on neurogliaform cells. The feedback inhibition, however, has not been linked with neurogliaform cells. In the present work, we aimed to shed light on the involvement of layer I GABAergic interneurons in the local microcircuits. We used in vitro acute brain slice electrophysiology combined with optogenetics and different specific transgenic mouse lines. Specifically, we investigated whether these neurogliaform cells receive excitatory inputs from the layer II pyramidal and stellate cells. Our results showed strong, monosynaptic excitatory connection from layer II pyramidal cells to neurogliaform cells. Moreover, we found that the properties of the EPSPs in neurogliaform cells elicited by layer II stellate cells are different from the EPSPs generated by layer II pyramidal cells. We hypothesize that these cells are involved in effective feedback inhibition of the entorhinal cortex microcircuits. Furthermore, we found that the neurgliaform cells are evenly distributed in layer I, therefore, they can convey inhibition in all cell types sending dendrites to layer I. Our research was funded by TKP20214. The research was performed in collaboration with the Nano-Bio-Imaging core facility at the Szentágothai Research Centre of the University of Pécs.

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Poster

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Topic: H.09. Spatial Navigation

Title: Differential reconfiguration of inter-neuron statistical dependencies in sensory and cognitive areas

Authors: *E. BALZANI, J.-P. NOEL, C. SAVIN, D. E. ANGELAKI;
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Abstract: Our ability to navigate across different environments remains relatively intact despite a broad range of sensory reliabilities. This is possible because neural circuits form stable percepts despite a dynamically changing sensory drive. However, the neural mechanism subserving such stable representations is still poorly understood. Here we record single unit activity from the dorsomedial superior temporal area (MSTd), parietal area 7a, and dorsolateral prefrontal cortex (dlPFC) as monkeys navigate in virtual reality to briefly presented target, akin to “catching fireflies”. The task requires the animal to sample from a close-loop visual environment while maintaining and continuously updating the representation of the distance to a memorized firefly location (the hidden spatial goal). We show that the monkey's performance is largely unaltered when sensory information in the form of optic flow is drastically reduced. We characterized neural responses via a Poisson Generalized Additive Model (P-GAM). This encoding model shows that MSTd tuning to task variables decreases under weak sensory reliability, while area 7a and dlPFC remain stable. Interestingly, MSTd increased the strength of putative lateral connectivity, estimated by inter-neuron statistical dependencies in the form of coupling filters, in the absence of reliable sensory information. Instead, 7a and dlPFC dynamically remapped their statistical dependencies. Further, the larger the remapping of coupling filters in 7a/dlPFC and the greater stability in MSTd, the less was the behavior of the monkey impacted by the lack of sensory evidence. At the population level, the remapping of dlPFC/7a resulted in more stable population dynamics when compared to MSTd. These results suggest that the remapping of lateral connectivity may play a role in maintaining stable neural representations to guide adaptive behavior in a changing environment.

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Poster

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Title: Navigation representations during active navigation are predominantly goal-directed

Authors: *T. ZHANG[1], J. L. GALLANT[2];
[1]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; [2]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: The human brain represents many different navigational features, such as scene identity, scene geometry, affordances, head direction, a cognitive, distances on the cognitive map, goals, planned routes, and progress towards a goal, among others. However, because most fMRI experiments study individual representations in isolation, the relative importance of these many different features to navigation remains unclear. To compare these representations, we developed an active navigation paradigm for fMRI. We built a 2×3 km virtual city populated by dynamic AI pedestrians and vehicles. In this city, subjects drove a virtual car using a set of MR-compatible steering wheel and pedals. Subjects learned the layout of the city prior to scanning. We used fMRI to record BOLD activity while subjects (2 male, 1 female, age 26-32) performed a taxi driver task in this world. On each trial, subjects were cued to drive to a destination. After subjects arrive, a new trial begins. Data were collected in 11-minute runs (110 min total for S1, 180 min total each for S2 and S3). Voxelwise modelling was performed with 21,283 stimulus- and task-related features that encompass 33 different types of information that might be represented during naturalistic navigation. Voxelwise models explain significant amounts of activity variance in 40.3% ± 7.8% of cortical voxels (mean ± std across subjects) voxels ($p < 0.01$) in each subject. Significant model predictions are found in many regions within and beyond known navigation-related ROIs, suggesting that active navigation is supported by broadly distributed networks in the brain. Visual and motor models account for over half the total explained variance (51.4%). In the navigational models, goal-directed representations account for the most variance. For example, the "future path" model accounts for 14.3 ± 6.0% (mean ± std across subjects) of the total explained variance. On the other hand, passive perceptual navigational representations explain vanishingly small amounts of the variance in voxel activity. For example, the "head direction" model accounts for 0.3% ± 0.6% of total explained variance. These data suggest that representations during active naturalistic navigation are predominantly goal-directed. It is likely that the 33 feature spaces used here do not encompass all possible features, and more models will reveal more fine-grained distinctions in their relative importance. Nevertheless, this study provides the most comprehensive description available currently of navigational representations in the human brain during active navigation.

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Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

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Program #/Poster #: 661.13

Topic: H.09. Spatial Navigation

Title: Decoding of spatial locations from primate lateral prefrontal cortex neural activity during virtual navigation

Authors: *R. JOHNSTON[1], M. ABBASS[2], B. W. CORRIGAN[2], J. C. MARTINEZ-TRUJILLO[3], A. J. SACHS[4];
[1]Univ. of Ottawa, Dunrobin, ON, Canada; [2]Univ. of Western Ontario, Univ. of Western Ontario, London, 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: Decoding of intended trajectories from brain signals using a brain computer interface system could be used to improve the mobility of patients with disabilities. Here we provide a proof of principle that multi-unit spiking activity recorded from the lateral prefrontal cortex of non-human primates can be used to predict the subject's location in a virtual maze during a navigation task. The spatial positions within the maze that require a choice, or are associated with relevant task events, can be better predicted than locations where no relevant events occur. Importantly, within a task epoch of a single trial, multiple locations along the maze could be independently identified using a support vector machine model. Considering that neurons in the lateral prefrontal cortex of macaques and humans have similar response properties, our results suggest this area could be a valuable implant location for an intracortical BCI system used for spatial navigation in patients with disabilities.

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Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 661.14

Topic: H.09. Spatial Navigation

Support: Flag-ERA VIPattract

Title: Neural activity of head direction neurons in the presubiculum in freely behaving mice

Authors: M. FERNANDA NIIÑO[1], J. GABILLET[1], *D. FRICKER[2], **M. GRAUPNER**[1];
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Abstract: Head direction (HD) neurons function as the brain's compass, forming our inner representation of the external world. The HD signal is internally generated, driven by vestibular information, and it is anchored to stable visual cues in the environment. How populations of neurons implement the stable maintenance of the HD signal and the dynamics upon

reorientation, is elusive. Our goal is to understand the dynamical structure of the spatial orientation network of neurons in the presubiculum and the dynamics for its resetting by salient visual cues.
To examine the underlying neuronal dynamics, we performed population recordings of presubicular HD cells using calcium imaging in freely behaving mice through a miniature fluorescent microscope. A round arena was used to control experimental recording conditions, i.e., switching between periods of light and darkness. Furthermore, the location of a prominent visual cue was dynamically changed to test if reorientation occurs following cue rotation.
Our results demonstrate that one photon calcium imaging can be used to determine HD tuning in populations of presubicular neurons. Specifically, we show that HD tuning of presubicular neurons is controlled by the location of the visual cue; is stable across same visual environments and is stable during darkness. We furthermore explore the relationship between spatial location of neurons and their HD tuning as well as the stability of the HD system across days. Our longitudinal imaging results of populations of presubicular neurons provide unprecedented insight in the internal dynamics and sensory control of HD neuronal activity.

Disclosures: **M. Fernanda Niiño:** None. **J. Gabillet:** None. **D. Fricker:** None. **M. Graupner:** None.

Poster

661. Neurophysiology of Spatial Navigation: Head Direction Cells and Cortical Circuits

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 661.15

Topic: H.09. Spatial Navigation

Support: ZIAAA000440

Title: Increasing activity within the medial temporal lobe rescues learning and memory deficits in the Scn2a mouse model of autism

Authors: J. A. MEZA, ***M. W. ANTOINE**;
NIH, Bethesda, MD

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder caused by genetic and/or environmental insults during prenatal development. In humans, haploinsufficiency of the *SCN2A* gene, which encodes the voltage gated sodium channel $Na_v1.2$, causes ASD that often co-presents with intellectual disability (ID). Typically, ID manifests as deficits in learning ability and episodic (recalling personal experiences), spatial, and visual memory. $Na_v1.2$ promotes ID via reductions to the sodium current which impairs the backpropagation of action potentials, reduces dendritic depolarization, and the postnatal expression of excitatory AMPA receptors that underlies plasticity mechanisms critical to learning. Here, we isolate brain regions and electrophysiological changes that cause the ID phenotype. Learning and memory were assessed with a spatial learning task where mice navigate a 20-hole maze to locate a single escape hole.

Over four days (with four 3-minute trials/day and an inter-trial interval of 30 minutes), wild type mice learn to preferentially adopt a spatial strategy to locate the escape hole rather than a serial strategy where each hole is sequentially searched. Juvenile/adolescent *Scn2a*$^{+/-}$ mice are severely deficient in their use of a spatial strategy, confirming a learning and memory deficit. Additionally, using an unbiased approach, we imaged and quantified cFos^{+} neurons in whole brains of *Scn2a*$^{+/-}$; *Fos-EGFP* mice to identify regions where basal neuronal activity is abnormal. We found significantly reduced numbers of cFos^{+} neurons in six brain regions, four of which are reciprocally connected within the medial temporal lobe (MTL) and functionally linked with memory impairments in humans, rodents, and primates. As these regions are cortical, we used the forebrain-targeting *Emx1*^{Cre} mouse line to conditionally delete *Scn2a* in excitatory pyramidal (PYR) neurons and replicated the spatial deficit phenotype. Furthermore, we restored and amplified spatial learning ability by chemogenetically increasing dendritic depolarization levels in excitatory PYR neurons of *Emx1*^{Cre}*;hM3Dq*$^{fx/+}$*;Scn2a*$^{+/-}$ mice. Thus, we show that by solely increasing depolarization within the MTL, ID symptoms in *Scn2a*$^{+/-}$ mice are reversed. Future experiments will identify transcriptional and protein expression differences that predispose MTL brain areas to functional impairment in cases of *Scn2a* deficiency. Altogether, this research identifies brain regions that may be pharmacologically targeted to yield improvement in ASD-associated learning and memory deficits.

Disclosures: J.A. Meza: None. **M.W. Antoine:** None.

Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Title: The Effect of Motor Memory Reactivation on Consolidation Depends on the Phase of the Stimulated Slow Oscillations: A Multimodal Neuroimaging Investigation

Authors: *J. NICOLAS[1], B. KING[3], D. LEVESQUE[4], L. LAZZOUNI[5], D. WANG[6], N. GROSSMAN[7], S. P. SWINNEN[8], J. DOYON[9], J. CARRIER[10], G. ALBOUY[2];
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London, Imperial Col. London, London, United Kingdom; [8]K.U.Leuven, K.U.Leuven, Leuven, Belgium; [9]McConnell Brain Imaging Ctr., McConnell Brain Imaging Ctr., Montreal, QC, Canada; [10]Hôpital Du Sacré-Coeur De Montréal, Hôpital Du Sacré-Coeur De Montréal, Montreal, QC, Canada

Abstract: Targeted memory reactivation (TMR) enhances sleep-dependent memory consolidation. Based on evidence that brain excitability fluctuates with the phase of sleep oscillations (SO), we hypothesized that the effect of TMR on memory consolidation depends on the stimulated phase of the SO. Sophisticated closed-loop TMR procedure was applied at two different SO phases (up vs. down) to test our hypothesis. Thirty-one healthy participants (age range: 18-30, 15 female) performed an audio-motor sequence learning task (three different motor sequences were learned) during which task-related brain activity was recorded with fMRI. Training and retest on the task were separated by a night of sleep during which closed-loop auditory TMR was applied while brain activity was monitored with EEG. Results show that TMR time-locked to the SO-down phase resulted in deterioration of performance speed as compared to up and no stimulation (N = 28; F (2,54) = 3.95; p = 0.034). fMRI data (N=28) indicate that, striatal activity increased from training to the overnight retest in both the up and down conditions (Up: Putamen right, Z = 5.12, p-uncorrected (p-unc) < 0.001; left: Z = 4.24, p-unc < 0.001; Caudate right: Z = 4.35, p-unc < 0.001; left: Z = 3.8, p-unc < 0.001; Down: Putamen right:, Z = 3.99, p-unc < 0.001, left: -32 0 2 mm, Z = 3.07, p-unc = 0.001; Caudate right: Z = 3.06, p-unc = 0.001). This effect was larger in the up as compared to the down (Putamen right: Z = 3.26, p-unc = 0.001; Caudate right: Z = 3.41, p-unc < 0.001) and not-reactivated (Putamen right: Z = 2.95, p = 0.002; Caudate right: Z = 2.86, p-unc = 0.002) conditions. In contrast, hippocampal activity decreased overnight in the not-reactivated condition (Hippocampus right: Z = 3.52, p-unc < 0.001; left: Z = 3.18, p-unc = 0.001) and this effect was significantly greater than in the up condition (right: Z = 3.08, p-unc = 0.001). EEG data (N=30) revealed phase-specific modulations of SO amplitude whereby up-stimulated SOs showed higher amplitude at the SO peak (cluster p-value = 0.002) and down-stimulated SOs 200 ms later (cluster p-value = 0.007). Additionally, sigma band power was significantly higher for the up- than the down-stimulated SOs during the ascending phase of the SO (cluster p-value = 0.008). Our results indicate that TMR effects depend on the stimulated phase of the slow oscillation. At the brain level, our data provide strong evidence for a phase-dependent modulation of (1) task-related brain activity in the hippocampus and striatum and (2) SO amplitude and sigma power; two processes known to play a critical role in sleep-related motor memory consolidation.

Disclosures: **J. Nicolas:** None. **B. King:** None. **D. Levesque:** None. **L. Lazzouni:** None. **D. Wang:** None. **N. Grossman:** None. **S.P. Swinnen:** None. **J. Doyon:** None. **J. Carrier:** None. **G. Albouy:** None.

Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Topic: H.10. Human Learning and Cognition

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Title: The stabilization of visual perceptual learning during REM sleep involves medial prefrontal cortex

Authors: *T. YAMADA, S. KHAN, P. KALYAN, P. SAGE, T. WATANABE, Y. SASAKI; Brown Univ., PROVIDENCE, RI

Abstract: Although it is well known that sleep facilitates visual perceptual learning (VPL), the neural mechanism underlying the sleep facilitatory effects was poorly understood. However, our recent research (Tamaki et al. Nat Neurosci, 2020) showed that early visual areas (EVAs) are greatly involved in the facilitatory effects shown as the E/I balance that refers to the concentration of excitatory neurotransmitter (Glx) divided by the concentration of inhibitory neurotransmitter (GABA) using magnetic resonance spectroscopy. In that study, we found that non-REM sleep plays a role in performance enhancement associated with an increased E/I balance in EVAs, whereas REM sleep stabilizes learning in association with a decreased E/I balance in EVAs. Another study found that reward provided during training both prolonged subsequent REM sleep and strengthens VPL after sleep (Tamaki et al., PNAS, 2020). This result suggests that that reward-processing circuits also play a role in some aspects of the facilitation. Given that the medial prefrontal cortex (mPFC) is in reward-processing circuits during sleep, here we investigated whether and how the mPFC plays a role in sleep facilitation of VPL.Young healthy subjects were trained on two different orientation discrimination tasks (TDTs) before and after a nap. These two TDTs were designed to interfere with each other, unless presleep learning was stabilized during sleep. During the nap, we simultaneously measured the E/I balance in the mPFC and polysomnogram to objectively determine sleep stages.We found that the performances of both pre-sleep and postsleep TDTs improved, indicating that pre-sleep learning was stabilized during the nap. As in our previous results of EVAs, the E/I balance in the mPFC decreased relative to baselines during REM sleep. However, the decreased E/I balance in EVAs was due to a decreased concentration of Glx, whereas that in the mPFC was because of an increased concentration of GABA.These results together suggest that although both mPFC and EVAs are involved in the stabilization of VPL during REM sleep, different neurotransmitters from different areas are coordinated. The mPFC may enhance and send inhibitory signals to reduce excitatory signals in EVAs during REM sleep for stabilization.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Program #/Poster #: 662.03

Topic: H.10. Human Learning and Cognition

Support: T32 MH112507

Title: Examining explicit motor learning mechanisms in normal aging: the temporal stability and savings of visuomotor adaptation

Authors: *J. A. KORTE, E. KRUSE, W. ZHOU, A. P. FAN, W. M. JOINER;
Univ. of California, Davis, Univ. of California, Davis, Davis, CA

Abstract: Normal aging is associated with a decline in learning and memory, which is also correlated with reduced motor performance. This study sought to combine fMRI imaging with a motor adaptation paradigm to gain insight into the neural changes that are correlated with age and distinct learning mechanisms. Specifically, we examined the extent the temporal stability of explicit learning mechanisms was directly related to its contribution to adaptation savings, and the correlation between motor performance and resting state connectivity assessed by fMRI. Motor adaptation studies are a tractable method to assess different components of learning and memory, and recent work has demonstrated implicit and explicit learning contributions to motor learning using a visuomotor rotation (VMR) task. This task measures motor planning and adaptation by inducing a rotational offset (e.g., 30^{o}) to visual feedback as the participant moves a cursor on a screen with an arm reaching movement. Explicit learning is quantified by the intended direction the subject selects prior to each movement, while implicit learning is derived from the difference between this selected movement and the actual hand movement. This task has been used to examine adaptation savings (faster relearning when the perturbation is experienced again following a break) and temporal stability (the retention of learning assessed by evaluating the amount of learning over a short time period, e.g., 5 to 90 seconds). Previous studies have used the VMR task to separately assess savings and temporal stability. However, possible relationships between the two learning properties have not been thoroughly examined. We analyzed the roles of implicit and explicit learning mechanisms in both tasks for healthy young (18-30) and elderly subjects (60 to 80+). In addition, using seed-based resting state fMRI, we examined how variations in learning for elderly subjects related to age-associated changes in hippocampal and cerebellar functional connectivity (structures associated with explicit and implicit components of motor learning, respectively). Our results may provide a basis for future clinical assessments of learning and memory deficits, specifically distinguishing the neural and behavioral changes associated with natural aging from neurodegenerative diseases (e.g., Alzheimer's Disease and Mild Cognitive Impairment).

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Topic: H.10. Human Learning and Cognition

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Title: The Influence of Attention on Implicit Sensorimotor Adaptation

Authors: T. WANG, ***J. LI**, R. IVRY;
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Abstract: Performance on sensorimotor adaptation tasks can be modulated by attention (e.g., Taylor and Thoroughman 2007). Manipulations of attention severely impact the use of explicit processes engaged in these tasks (Haith et al, 2016). However, the impact on implicit processes is less clear: Adaptation occurs automatically in response to task-irrelevant clamped feedback (cursor position independent of hand position), even when participants are instructed to ignore the clamped feedback. This makes clear that adaptation can appear when the feedback is task-irrelevant and unattended. However, it remains possible that implicit adaptation is influenced by attentional state. One recent study (Morehead et al, 2022) used dual task-contingent feedback (cursor position yoked to hand position), with the participants instructed to compensate for the error based on just one of the cursors. Implicit adaptation was much larger in response to the task-relevant feedback, perhaps because participants paid more attention to this cursor. While this result would suggest that implicit adaptation is influenced by spatial attention, it is also possible that implicit adaptation is impacted under divided attention conditions, perhaps subject to constraints associated with cognitive resources.
Here we manipulated the demands on spatial attention and cognitive load in a series of visuomotor adaptation experiments. To manipulate spatial attention, we used a dual-task procedure in which participants reached to a visual target while concurrently performing an orientation discrimination task. For the latter, two bars were presented, one on each side of the target and a central cue indicated the task-relevant bar for the current trial. Clamped feedback (rotated 15° from the target) appeared on the cued or uncued side. Adaptation to the clamp was of comparable magnitude when presented on the cued or uncued side. We confirmed this result in subsequent experiments using different methods to manipulate spatial attention; as such, we failed to find evidence that implicit adaptation could be modulated by spatial attention. However, the magnitude of adaptation was considerably lower (~75%) in the dual-task condition compared to the single-task condition in which the participants were instructed to ignore the bar (and ignore the feedback cursor). A similar suppressive effect was observed under dual-task conditions when the bar appeared at the target. Thus, the inclusion of a secondary task had a large suppressive effect on implicit adaptation to clamped feedback. Taken together, these results show that manipulations of cognitive load but not spatial attention impact implicit adaptation.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.05

Topic: H.10. Human Learning and Cognition

Title: The effect of the time-of-day on motor skill consolidation

Authors: *C. TRUONG, C. RUFFINO, J. GAVEAU, C. PAPAXANTHIS;
INSERM UMR1093-CAPS, Univ. of Burgundy, Dijon, France

Abstract: The passage of time impacts motor memory consolidation[1]. Recently, we have shown that this effect depends on the time-of-day, notably with a deterioration of skill 24 hours after a morning training[2]. Here, we tried to understand the underlying mechanisms of this deterioration. Twenty-four healthy adults were trained on a finger tapping task (speed-accuracy trade-off task), either at 10 a.m. (n = 12) or 3 p.m. (n = 12). The training was composed of 48 trials (6 sequences of 6 keys per trial). To evaluate motor performance improvement, we analyzed the skill (i.e., the ratio between duration and accuracy) of the first two and the last two trials. To evaluate motor memory consolidation, participants were retested in two trials 5 hours after the end of the training. Although both groups similarly improved their skill performance after the training, there was a deterioration in consolidation (5 hours after the training) only for the morning group (10 a.m.); the performance of the afternoon group (3 p.m.) was stabilized. To further investigate this finding, we included 24 others participants in two similar groups (training at 10 a.m. vs 3 p.m.). The experimental protocol was the same, with the difference that we added an interference task (i.e., second training with another sequence of finger tapping) immediately after training to test the robustness of motor memory. Both groups similarly improved their performance after the training of the first as well as the second sequence. Interestingly, the skill consolidation was significantly deteriorated for the morning compared to the afternoon group, revealing a more labile memory after a morning training. As the quantity of physical activity differs from the morning to the afternoon, we tested whether an increase in physical activity before the training session of the morning group could preserve motor consolidation. We divided 24 others participants into two groups which were trained at 10 a.m., one was physically active prior to training. We retested participants 10 hours later. While we found a deterioration of performance for the group without physical activity, the group with physical activity stabilized the performance 10 hours after the training. Overall, it seems that the passage of time deteriorates motor skill consolidation when the training takes place in the morning. A daily activity prevents this deterioration. These findings identify an important factor to consider for optimizing training programs or rehabilitation protocols in the sport and clinical domain. [1]Robertson, Pascual-Leone & Miall (2004). Nature Reviews Neuroscience. [2]Truong, Hilt, Bouguila, Bove, Lebon, Papaxanthis & Ruffino (2022). Scientific Reports

Disclosures: **C. Truong:** None. **C. Ruffino:** None. **J. Gaveau:** None. **C. Papaxanthis:** None.

Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

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Program #/Poster #: 662.06

Topic: H.10. Human Learning and Cognition

Title: Learning-related corticospinal plasticity is sensorimotor mu phase-dependent

Authors: *T. SURESH, F. IWANE, M. ZHANG, S. J. HUSSAIN;
Univ. of Texas, Austin, Univ. of Texas, Austin, Austin, TX

Abstract: The sensorimotor cortex plays a critical role in motor learning. Recent studies show that transcranial magnetic stimulation (TMS) applied to the primary motor cortex (M1) preferentially induces long-term potentiation-like changes in corticospinal output when delivered during mu rhythm trough phases. Furthermore, TMS applied during mu trough but not peak phases boosts motor learning. Together, these studies suggest that motor learning may preferentially occur during mu trough phases. If so, learning-related corticospinal plasticity should also be mu phase-dependent. To address this possibility, we recruited healthy right-handed adults for a study involving TMS, EEG, EMG, and behavioral assessments. Participants were randomly assigned to either a repetition or no-repetition group. Using their right hand, the repetition group practiced 14 blocks (120 trials per block) of the implicit serial reaction time task (SRTT), which contained an embedded, repeating 12-item sequence. The no-repetition group practiced a control version of the SRTT using their right hand; this version contained no sequence. We delivered single-pulse mu phase-dependent TMS (intensity=120% of resting motor threshold) to the left M1 before (baseline), immediately after (Post0), and 30 minutes after (Post30) the SRTT task. To evaluate retention, the repetition group performed a shorter version of the implicit SRTT one hour after initial practice, while the no-repetition group performed a shorter version of the control SRTT one hour after practice. Preliminary analysis (N = 5 per group) revealed that real-time targeting of mu peak, trough, and random phases recorded over the left sensorimotor cortex was accurate. As expected, the repetition group showed more sequence-specific learning than the no-repetition group during initial practice and retention. In the repetition group, MEP amplitudes increased after SRTT practice. However, mu phase-dependency of MEP amplitudes was present immediately after SRTT practice but not at baseline or 30 minutes after the SRTT. At Post0, MEPs were largest at mu troughs. In contrast, participants in the no-repetition group showed no significant change in MEP amplitudes over time but did show similar phase-dependency at all time points. MEPs were largest at mu troughs regardless of time point. These early results suggest that learning-related changes in corticospinal output are most strongly expressed during mu trough phases, consistent with a phase-dependent motor learning mechanism.

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Poster

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Topic: H.10. Human Learning and Cognition

Support: NSF-DRL 1631563

Title: Individual variation in the lateralization and exaptation of toolmaking neural circuitry for language use

Authors: *S. VIJAYAKUMAR[1], S. PEZZULO[1], S. SINGH[2], C. M. CONWAY[3], J. PARGETER[4], N. KHREISHEH[5], D. STOUT[6], E. E. HECHT[1];
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Abstract: The brain has acquired the ability to coordinate hierarchical, increasingly complex sequential behavior, such as toolmaking and intentional communication, due to increased lateralization within the primate lineage. Given the overlap of brain regions used in toolmaking and language tasks, and archeological evidence indicating the evolution of toolmaking preceded the onset of language in the hominin lineage, it is likely that the neural architecture used in language was exapted from the brain regions used in toolmaking. However, there has been little research examining whether variation in the extent of lateralization of language pathways is associated with differences in language and toolmaking ability within an individual. To address this gap, we recruited 37 participants, aged 18-49 years (mean = 29.3, s.d. = 8.9, 28 female). T1 scans and diffusion scans were acquired using a Siemens 3T Trio scanner. We then performed probabilistic tractography to identify the two major white matter tracts implicated in toolmaking and language use -- superior longitudinal fasciculus II/III (SLF-II/III) and the arcuate fasciculus (AF), respectively. Toolmaking ability was evaluated using methods grounded in experimental archaeology reflecting participants' perceptual motor accuracy, accuracy of predicting strike outcomes, and strategic reasoning ability. Language ability was evaluated using an artificial grammar learning (AGL) task and a syntactic simplicity score derived using the Coh-Metrix 3.0 tool, based on participants' written statements of approximately 500 words. Statistical analyses using Benjamini-Hochberg correction for multiple comparisons found that left lateralized gray matter termination volume of AF in BA44, BA45, and the temporal lobe was significantly correlated with one's ability to learn frequently encountered sequences of letters in the AGL task. Also, left lateralized gray matter termination volume of SLF II/III in the inferior parietal lobe correlated with one's ability to predict strike outcomes during toolmaking. Additionally, toolmaking ability was significantly correlated with the volume of tract terminations exclusively in key regions of the brain involved in phonological processing and other language abilities. Together, our findings are consistent with the view that an increased circuit lateralization contributed to the evolution of the capacity for complex, hierarchically organized behavior used in both language and toolmaking.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Program #/Poster #: 662.08

Topic: H.10. Human Learning and Cognition

Title: Examining childhood advantages in the learning and consolidation of movement sequences

Authors: *A. VAN ROY, B. R. KING, G. ALBOUY;
Hlth. and Kinesiology, Univ. of Utah, SALT LAKE CITY, UT

Abstract: Abstract Motor development research is often grounded in the notion that young adults are the model of optimal functioning and children are thus conceptualized as developing systems progressing towards this ideal state. Although this framework certainly has its merits, there is also some evidence suggesting that children can outperform young adults in specific motor learning-related behaviors. For example, whereas motor memory consolidation can be considered a protracted process that requires time and sleep in adults, children have shown rapid consolidation of a motor sequence following 1 hour of post-learning wakefulness. Such a developmental advantage does not appear to be limited to the timescale of hours following practice (i.e., referred to as "macro-offline"), but is also evident on the timescale of seconds during the rest periods between practice blocks (i.e., "micro-offline"). This previous research, however, does have limitations, including the lack of direct comparisons to young adults and/or small sample sizes, that prevent the formation of strong conclusions. The current research thus aimed to systematically examine these potential childhood advantages in motor learning and memory consolidation behaviors. Behavioral data were acquired from 149 7- to 35-years-olds across two experiments that required participants to perform between 1-3 sessions of a bimanual serial reaction time task (SRTT) via an online data acquisition platform. Results revealed that the magnitude of micro-offline performance gains during early learning was significantly greater in 7- to 12-year-old children as compared to 18-35-year-old young adults ($t_{(39.715)} = 2.973$, $p = 0.005$). Moreover, children showed higher macro-offline gains for both the five- and twenty-four-hour retest sessions. However, this difference did not reach significance in this preliminary sample. Collectively, these results suggest that children may exhibit a developmental advantage in the offline processing of recently practiced motor sequences.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Economic and Social Research Council-funded South West Doctoral Training Partnership [training grant reference ES/P000630/1]

Title: Grasping mental fatigue: developing a novel method to induce mental fatigue in the context of visuomotor control and dexterity

Authors: *E. K. HASSAN, A. M. JONES, G. BUCKINGHAM;
Univ. of Exeter, Exeter, United Kingdom

Abstract: Being able to effectively coordinate the fine movements of our arms, hands, and fingers is an essential skill that enables us to complete daily tasks safely and efficiently. This ability may, however, be affected by an individual's physical and mental status. Mental fatigue is a commonplace experience which is the focus of a growing body of research. Whilst researchers in numerous disciplines have attempted to uncover the origins, nature, and effects of mental fatigue, the literature is marked by many contradictory findings. The effects of mental fatigue on hand function are particularly unclear. This lack of clarity is due to conceptual and methodological differences and, in some cases, a lack of scientific rigour. We identified two major methodological problems for research aiming to elucidate the effects of mental fatigue on hand function. First, researchers rarely use objective measures of mental fatigue, relying instead on subjective reports as evidence that mental fatigue has been induced in participants. We aimed to develop a task which led to both a subjective increase in mental fatigue, and an objectively measurable performance decrement in the mentally fatiguing task. Secondly, current mental fatigue paradigms have low ecological validity - in most prior studies participants have been fatigued with a single repetitive task such as the N-Back or Stroop. To move towards a more ecologically valid paradigm, we designed a two-hour battery of diverse cognitive tasks designed to challenge different aspects of executive function. We report results from 46 participants aged 20-63 who completed this battery. Participants' subjective fatigue ratings and task performance on the A-X Continuous Performance Test were measured at the beginning and end of the tasks. Our preregistered analyses found that our novel method resulted in both an increase in subjective ratings of fatigue ($p < 0.001$, $r = 0.85$) and a reduction in task performance ($p = 0.006$, $r = 0.39$). We conclude that our novel method is suitable for inducing mental fatigue in future experimental research.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.10

Title: WITHDRAWN

Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.11

Topic: H.10. Human Learning and Cognition

Title: Sequence learning across memory domains

Authors: *A. TEMUDO[1], N. DOLFEN[3], A. DORNIER[4], C. WILLIAM[1], B. R. KING[1], G. ALBOUY[2];
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Abstract: Abstract: Memory systems do not act independently but interact. There is a plethora of evidence that the behavioral and neural correlates of sequence learning are similar across memory domains. However, such between-memory-domains similarities have never been empirically tested. The goal of the present study was therefore to address this knowledge gap. To do so, we designed a new version of the serial reaction time (SRT) task using pictures of objects as visual cues to trigger motor responses. Three different versions of the task were designed such that participants would either (1) learn a sequence of objects associated to random key presses (object sequence task), (2) learn a sequence of finger movements associated to a random presentation of objects (motor sequence task) or (3) practice a random (key and object) version of the task that was used as a control. 13 young healthy participants (age range: 18-30, 9 female) learned both the motor and object sequence tasks 4 hours apart. Consolidation was assessed with a functional magnetic resonance imaging version of the tasks during an overnight retest.
Behavioral analyses of performance speed (response time) performed on the training data indicated that participants learned both the object and motor sequences (block effect: $F(19,228)=20.93$, $p<0.001$) to the same extent (condition effect: $F(1,12)=2.87$, $p=0.12$; condition by block effect: $F(19,228)=1.20$, $p=0.26$). Data acquired during an immediate post-training test showed that performance plateaued (block effect: $F(3,30)=1.54$, $p=0.22$) similarly between conditions (condition effect: $F(1,10)=0.54$, $p=0.48$; condition by block effect: $F(3,30)=0.27$, $p=0.85$). During the overnight retest, performance further improved (block effect: $F(3,30)=6.63$, $p=0.001$). Importantly, performance on sequence and random tasks differed (condition effect: $F(2,20)=22.04$, $p<0.001$) such that both sequence tasks were performed faster than the random task (object vs. random: $p=0.002$; motor vs. random: $p<0.001$) and performance was similar between sequence tasks (object vs. motor: $p=0.17$).
Our preliminary results indicate that this new version of the SRT task allows participants to learn sequences of movements and objects to the same extent. They also show similar retention of the

sequence-specific knowledge across memory domains during the overnight retest. Representational similarity analyses of the corresponding neuroimaging data will unravel the neural processes supporting sequence learning across memory domains.

Disclosures: **A. Temudo:** None. **N. Dolfen:** None. **A. Dornier:** None. **C. William:** None. **B.R. King:** None. **G. Albouy:** None.

Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.12

Topic: H.10. Human Learning and Cognition

Title: Set-size effect in automatic action selection

Authors: *Y. DU, A. M. HAITH;
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Abstract: Action selection is often slower when a task involves a wide range of stimuli and responses than when there is only a small number of stimulus-response alternatives. This set-size effect on response time has been well established, however, only when action selection is driven by cognitive processes such as computation and memory retrieval (Proctor & Schneider, 2018). The set-size effect does not seem to hold for very well-learned skills such as making a reaching movement or saccade towards a spatial target (Proctor & Schneider, 2018). This observation is well aligned with existing theories of skill learning that practice leads to a qualitative change in how we perform a task, marked by a change from being initially deliberative or cognitively demanding, to automatic or habitual (Fitts & Posner, 1967; Haith & Krakauer, 2018). According to this view, automatic or habitual action selection is generally conceptualized as a reflex-like direct stimulus-response association (Du, Krakauer, Haith, 2022), and thus ought to be immune from the effect of set size. However, recent studies reported that our behaviors could become habitual quite early in learning when we are still relatively unskilled (Yang, Cowan, Haith, 2022; Hardwick, Forrence, Krakauer, Haith, 2019). As such, one would expect to observe the set-size effect on automatic or habitual action selection. Here, we aim to examine these two opposing hypotheses. We ask participants to perform the same non-arbitrary visuomotor association task that consists of eight stimuli (i.e., numbers 1-4 and letters a-d) mapping to eight keys in rank order. We then use a free-reaction time task to examine the set-size effect on response time by assessing how fast participants could respond to the full set of stimulus-response association and a reduced size stimulus-response association with only four stimuli and responses (e.g., two numbers and two letters, randomly selected for each participant; the order of these two associations is counterbalanced across participants). Because free response time may not reflect the minimum possible time at which a response can be initiated (Haith, Pakpoor, Krakauer, 2016), we further examine whether the set-size effect is present in action selection using a timed-response approach. Lastly, we confirm whether action selection becomes habitual by asking

participants to revise their responses to two pairs of stimuli (Du & Haith, 2021). Results from this study will reveal the nature of habitual/automatic action selection regarding whether it is like a simple reflex or it involves cognitive processes such as memory retrieval.

Disclosures: Y. Du: None. **A.M. Haith:** None.

Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.13

Topic: H.10. Human Learning and Cognition

Support: NSF-DRL 1631563

Title: Individual variation and prior experience shape neuroplasticity during Paleolithic stone toolmaking skill acquisition

Authors: *E. HECHT[1], J. PARGETER[2], N. KRIESHEH[3], D. STOUT[4];
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Abstract: The cultural transmission of complex technological skills is one of the traits that sets humans apart from other animals. Paleolithic stone toolmaking is one such skill which is likely to have shaped the evolution of the human brain. In this study, we used a combination of diffusion-weighted MRI and archaeologically-grounded Paleolithic stone toolmaking training to examine white matter plasticity in 17 experimental participants and 16 controls. Tract-based spatial statistics revealed that pre-training toolmaking skill was associated with individual variation in fractional anisotropy within prefrontal voxels that connect the left inferior frontal gyrus with left lateral temporal regions via ventral pathways likely including the extreme capsule and uncinate fasciculus. Additionally, we observed plastic change in voxels of the right ventral premotor cortex and right inferior frontal gyrus which connect with right inferior parietal cortex via the third branch of the superior longitudinal fasciculus (SLFIII). These results confirm the importance of fronto-temporal-parietal networks, particularly right SLFIII, in Paleolithic stone toolmaking, indicating that adaptations to this network were likely crucial for evolved increases in technological complexity. Additionally, individual variation in participants' prior experience with crafts like carpentry and sculpture significantly predicted both toolmaking performance and brain measurements. This suggests that links between prior skill learning and acquired brain change may have played an important role in the cultural transmission and proliferation of new technological skills during human evolution.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Topic: H.10. Human Learning and Cognition

Support: NIH K01HD093838

Title: Explicit exploration during virtual throwing and skill transfer to a real-world task in healthy children and young adults

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Abstract: Motor exploration during skill acquisition may help the motor system identify the consequences of different strategies and find new or better solutions, potentially facilitating motor learning. This study investigated the impact of “explicit” exploration, where participants were instructed to explore alternative strategies after having reached a pre-determined success threshold. Using a virtual throw-to-target task, we tested its effect on task performance during acquisition, retention, transfer to a new target location and to an equivalent real-world task. Given the redundancy of the virtual task, infinite angle-velocity combinations at ball release are possible to achieve a given target hit or error (minimum distance between ball and target). To encourage further exploration following a pre-determined threshold, a virtual obstacle was introduced that blocked participants from continuing to use the same release variables as previously. 84 healthy participants performed 175 throws (acquisition) either in the exploration or control condition (no obstacle was inserted). They returned 1-3 days later to perform 25 throws in the same condition as acquisition (retention), followed by 50 throws with the target at a new location (close transfer), and 50 throws in a real-world setup whose parameters were identical to the virtual task (far transfer). Mixed-effects models evaluated changes in session median error between conditions. Decomposition of variability in throw variables within each session used the TNC-method, quantifying Tolerance, Noise, and Covariance components. Participants in the exploration condition demonstrated greater error during acquisition and retention sessions compared to control. No differences in performance were observed between conditions in either close or far transfer sessions. In the close transfer session, exploration participants had higher T- and C-costs compared to control participants, indicating that short-term practice under explicit exploration conditions does not help to identify more good solution regions nor exploit the redundancy of the execution space. Explicit exploration in a virtual environment showed no benefit for transfer or transfer to a real-world skill, suggesting that exploration as a learning strategy relates more to intrinsically-motivated rather than externally-

imposed practice. Subsequent studies will further evaluate the impact of intrinsically-motivated exploration on motor skill acquisition and transfer.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Program #/Poster #: 662.15

Topic: H.10. Human Learning and Cognition

Support: NSERC

Title: Exploring the relationship between sensory gating, task-relevancy, and motor learning.

Authors: K. E. BROWN, L. B. KAETHLER, *W. R. STAINES;
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Abstract: Motor learning is dependent on somatosensory information to guide movement and provide feedback. The somatosensory system can be regulated based on task-relevancy. Relevant information is facilitated while irrelevant information is inhibited. Despite the importance of somatosensory modulation, how it is employed across different stages of motor learning is not fully understood. The aim of the current work was to understand how relevant and irrelevant somatosensory information were modulated across a motor sequence task. We hypothesized that early in learning there would be an increased dependence on somatosensory feedback to enhance improvement, which would result in greater facilitation of relevant and inhibition of irrelevant somatosensory information. Conversely, once individuals' performance plateaued, we expected less modulation of incoming afferents. To test this, 20 healthy young participants completed an experimental session in which somatosensory-evoked potentials (SEPs) were collected while they performed a motor sequence task. During the task, vibration was delivered to the index finger of the non-dominant hand. Vibration amplitude was changed across a 30 s time-window and participants were asked to match the amplitude changes by squeezing a pressure-sensitive bulb with their dominant hand. Visual feedback was provided after each 30 s sequence. The sequence task was repeated 32 times, or until performance plateaued. This procedure was then repeated with a random, unlearnable sequence to enable comparison between motor learning and motor control. During the task, SEPs were evoked from ring electrodes on the index (task-relevant) and pinky (task-irrelevant) fingers of the nondominant hand. SEPs were also taken at rest. To process SEP amplitudes, the behavioural task was divided into four components: Early/Late Repeated; Early/Late Random. SEPs were epoched from -100 to 300 ms and averaged in each of these time-windows and in the Rest condition. SEP amplitudes (N20, P26, N30, P50, N70, P100, and N140) were then quantified from the electrode over the contralateral somatosensory cortex (CP3/4). Results from a 2 x 2 repeated-measures ANOVA showed a Time

x Finger interaction, such that N30-P50 SEP amplitudes were only modulated in the index, or task-relevant, finger. Post-hoc tests reveal that somatosensory information is facilitated early in the repeated task, but this is not seen when the pattern is random. These results suggest that somatosensory information is differentially modulated across stages of motor learning, such that task-relevant information is facilitated to potentially promote early performance improvements.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

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Program #/Poster #: 662.16

Topic: H.10. Human Learning and Cognition

Title: Predicting math abilities from resting-state functional connectivity using Polyneuro Risk Scores.

Authors: *A. VAN RINSVELD[1], N. BYINGTON[3], G. GRIMSRUD[4], M. MOONEY[7], M. CORDOVA[8], O. DOYLE[7], R. J. HERMOSILLO[5], E. EARL[7], A. PERRONE[3], L. A. MOORE[3], A. GRAHAM[9], J. NIGG[7], W. THOMPSON[10], E. FECZKO[11], M. GUILLAUME[1], E. ROY[1], O. MIRANDA DOMINGUEZ[4], D. A. FAIR[6], B. D. MCCANDLISS[2];
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Abstract: Math ability is one of the most complex human cognitive skills, but also a large source of inter-individual variability that has an important influence on life outcomes. Math learning is overlapping with domain-general skills and language across development. Prior research has identified several brain regions linked to math abilities but the specificity of those circuits for math has not been systematically assessed. This makes it difficult to build a unified model of the functional brain network supporting mathematical skills. We tested whether efficient math skills are predicted by a distributed or focal set of functional connections across the brain, and whether those connections are specific for math or generalize across several high-order cognitive functions. We leveraged a large sample of participants (N=2749) from the Adolescent Brain Cognitive Development (ABCD). Resting-state functional connectivity (RSFC) data were collected at the baseline of ABCD (ages 9-10) and math abilities were assessed three years later (ages 12-14). We used a BWAS approach to calculate polyneuro risk scores (PNRS) of math based on the weighted contribution of distributed, whole-brain functional connectivity and contrasted the performance of the whole brain PNRS to models per functional circuits. This approach aimed at identifying the functional connections predicting efficient math skills. We calculated a linear mixed model on half of the sample and applied the outputs to the

second half (demographically matched split-halves) to calculate a PNRS that was then compared to the actual behavioral outcome. Performance of the predictions were compared with and without controlling for domain-general cognitive skills. Results showed that few connections belonging to specific networks explain most of the variance in math skills. The maximum explained variance was reached with 3 network pairs corresponding to 7% of brain coverage. These findings suggest that networks supporting math skills are associated to focal effects rather than being distributed across the entire brain. Unlike recent application of BWAS approaches to other cognitive domains, functional connections supporting math abilities seem to be very focal. Math efficiency was better predicted when domain-general functions were accounted for in the model, despite the latter being highly correlated to math. This strongly supports the specificity of the functional connectivity features supporting math abilities and highlights an effective model of calculating polyneuro risk scores to predict math efficiency from functional connectivity in a longitudinal setting.

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Poster

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Topic: H.10. Human Learning and Cognition

Support: This study was conducted as part of Global Singularity Research Program for 2022 financially supported by KAIST.

Title: Information-theoretic understanding of the prefrontal principle in fast performance improvement during sensorimotor adaptation

Authors: *Y. SONG[1], J. JEONG[1,2];
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Abstract: Humans show flexible and versatile motor learning ability, which stems from the remarkable ability of the brain to adapt to new environments. The prefrontal cortex is an important candidate region that enables the brain's fast and flexible motor adaptation (MA) process (McDougle et al., 2016), but the neural principle of such a process is still elusive. Therefore, we sought to understand the underlying neural principle of the brain's fast MA process using information theory and MRI analysis techniques. We first theoretically formulated

the MA process as two mappings of random variables: one from MA input (e.g., sensory information or motor commands) to internal representation (IR; indicating neural activities in any MA-related areas, such as the prefrontal area or cerebellum), another from the IR to MA output (e.g., actual movements). From this, we derived the following prediction: learning speed (i.e., how fast MA is conducted) positively correlates with the cardinality of the IR (i.e., how much information the MA system can internally represent). To verify this, we conducted the brain-imaging experiment (n=47, female=22, mean age=23.7, s.d.=2.6) with a newly designed MA task where the participants need to erase given stimuli by adapting to the horizontally reversed computer mouse, and found that the participant's learning speed during the task indeed positively correlates with the gray-matter volume of the right DLPFC (which reflects the cardinality of the information coded in that area) ($p\sim0.0088$, corrected for the whole brain, cluster level), as predicted in the theory. This suggests that the right DLPFC is crucial in coding the input-output mapping required in MA. We further found that BOLD activation in the right DLPFC is associated with instantaneous performance improvement (i.e., trials when MA performance is largely increased compared to the previous trial) ($p\sim0.0062$, small-volume correction, cluster level), supporting the right DLPFC role as a fast MA process. Lastly, based on the previous observation (Wang et al., 2018), we speculated that the DLPFC shows faster learning than the other MA areas (e.g., cerebellum) by meta-controlling the other MA process (e.g., the motor loop through the cerebellum). Indeed, our theoretic description showed that such a meta-process can exhibit faster DLPFC learning. Also, dynamic causal modeling showed that the right DLPFC exerts control over the cerebellum (posterior > 95%), well-matched with our suggested relational structure of the DLPFC (as a meta-controller). In conclusion, the current work identifies the critical characteristics of DLPFC as a fast MA system and the determinants of fast MA learning.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Topic: H.10. Human Learning and Cognition

Support: SFB874/A11

Title: The flexibility of implicitly versus explicitly learned motor sequences

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Abstract: We learn and in turn use motor sequences on a daily basis. Learning a motor sequence, for example a dance choreography, consists of two processes: Acquiring knowledge about the sequence elements and their temporal order (explicit component) and being able to

combine these elements into a single, skilled behavior, i.e. to perform the sequence fluently (implicit component). On occasion, it is necessary to correct an already learned motor sequence, i.e. to exchange an incorrectly learned element of the sequence. For example, one might have learned a dance choreography including a wrong step over several weeks, and be confronted with re-learning the choreography incorporating the correct dance step. Motor sequence learning can be either implicit or explicit, while the relationship between both learning systems is not fully understood. In this study, we want to investigate how implicitly or explicitly learned sequences adapt to corrections, while our hypothesis is that the latter are more flexible in terms of re-learning. In our task design, participants concurrently learn an implicit and an explicit motor sequence of key presses in a modified version of the serial reaction time task (Nissen & Bullemer, 1987). The participants undergo five training sessions over the course of a week, while EEG recordings are performed at the first and last training session using a wet 64-electrodes EEG system. After the last training session, the stimulus-response mapping is changed, resulting in new, yet highly overlapping motor sequences to the ones previously learned. In this way, the correction of an extensively trained sequence is mimicked. Using a concurrent learning task design allows us to investigate how flexible implicitly and explicitly learned motor sequences adapt to a correction in an intra-subject manner. We will present our study design and data of 10 participants, including behavioral as well as EEG data. On a behavioral level, implicit and explicit motor sequence learning is reflected by significantly reduced reaction times compared to a control condition, while the reaction times are increased in the re-learning condition. A time-frequency analysis of the EEG data shows that especially beta power suppression at motor-cortical electrodes plays a role in motor sequence learning. The beta power suppression is greater in the explicit condition and we conjecture it to be an indicator of suppressing the current motor and cognitive state in favor of flexible control strategies. **References:** Nissen MJ & Bullemer P (1987). Attentional requirements of learning: Evidence from performance measures. *Cognitive Psychology, Volume 19, Issue 1, January 1987, Pages 1-32.*

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Topic: H.10. Human Learning and Cognition

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Title: Functional contributions of brain activity to motor skill learning

Authors: *I. GUTIERREZ, N. FIRESTONE, R. KIRBY, C. MCCARTHY, N. BAUNE, J. MIRDAMADI, M. BORICH;
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Abstract: The premotor cortex (PMC) equivalent in animals is essential for sequence-specific motor skill acquisition while the role of PMC in humans remains unclear. Transient interference of cortical activity with transcranial magnetic stimulation (TMS) can be used to evaluate the necessary role of brain regions in behavior. Previously, we observed that sequence-specific motor skill acquisition was not observed following TMS-based interference of either the PMC or primary motor cortex (M1) during training. The lack of a sham TMS control and retention testing limits interpretation. In the current study, we hypothesized that sequence-specific skill acquisition following motor skill training with sham TMS would occur and that higher task difficulty would result in greater skill retention. 39 neurotypical, right-handed young (18-36 years old) adults were recruited. In experiment 1, participants were randomly assigned to receive TMS to PMC (N=9) or M1 (N=10) delivered during a single training session on a modified serial reaction time task (SRTT). In experiment 2, a separate cohort of participants received sham TMS during training with either a higher (N=10) or lower (N=10) level of difficulty. After 24hrs, participants returned for retention testing in experiment 2. General motor performance was assessed as by response times on random sequences. A skill score (SS) was calculated as the difference in repeated vs. random sequence performance. Motor skill acquisition was assessed as change in SS between post- and pre- training blocks. Motor skill learning was quantified as the difference in SS between 24hr and post-training. TMS-based stimulus-response curves indexed M1 cortical excitability before and after SRTT training. One-way analysis of variance and t-tests were performed for each outcome measure. Change in general motor performance after training was lower in the M1 TMS group compared to sham TMS ($p=0.03$). Motor skill acquisition was not different between stimulation ($p=0.7$) or task difficulty groups ($p=0.4$). Great motor skill learning was observed for the higher task difficulty group ($p< .0005$). Reduced excitability in the sham TMS group ($p=0.05$) was observed. Findings demonstrate training-related improvement in general SRTT performance that was reduced by M1 interference, but effects were not sequence-specific. These results support the role of M1 in motor execution rather than sequence-specific skill acquisition. Our hypothesis that PMC has a necessary role in sequence-specific skill learning requires further investigation due to the lack of retention testing performed in the first experiment given learning was shown after a single training session.

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Poster

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Topic: H.10. Human Learning and Cognition

Support: NSF BCS2011716

Title: Mobile Brain/Body Imaging of three-ball juggling: Dynamics of neurobehavioral interactions between motor execution and perception

Authors: *J. IVERSEN[1], H. KIM[1], M. MIYAKOSHI[1], H. KAMBARA[2], H. TANAKA[3], T. KAGAWA[4], M. SATO[5], S. MAKEIG[1], N. YOSHIMURA[6];
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Abstract: We aim to develop a neurobehavioral model of complex skill learning by integrating information from brain activity and body movement in real time. We use three-ball juggling, a task requiring precise coordination between visuospatial processing and inter-limb motor execution. There has been relatively little study of the neural dynamics of such a complex motor act. We have developed methods to jointly analyze movement and brain activity and present results from the analyses on thirteen jugglers with a range of skill levels. We first examined motion capture and ball trajectories to characterize juggling in terms of three components of juggling skill: throw accuracy, ball trajectory prediction, and timing stability. Throw accuracy, measured by the variability of ball apex position, was not correlated with juggling skill (indexed by mean number of juggling cycles before dropping a ball), although the variability of accuracy (cycle to cycle consistency of error) clearly was. High-skill jugglers were most clearly characterized by timing stability in ball throws, while timing variability in ball catches was comparable in all jugglers, suggesting tight control of throws and the absorption of cycle-to-cycle variability by the timing of catches. Brain dynamics were assessed using spectro-temporal analysis of cortically resolved high-density scalp EEG referenced to timing events extracted from motion capture data. We demonstrate that it is possible to reliably extract spatially localized brain activity related to visual processing, spatial attention, multi-sensory integration and motor execution despite movement associated with juggling. In parietal cortex, known to be involved in spatial processing, we found robust alpha-band desynchronization at the moment the thrown ball reached its apex, a time thought to be critical for trajectory estimation required for the planning of the timing and location of the next catch. Motor regions had activity correlated to contralateral hand movements, with broad-band increases around the time of catch. A notable finding is very narrow-band activity between 70-80 Hz that shows periodic modulation with positive/negative peaks corresponding to contralateral/ipsilateral throws and sharp transitions corresponding to catches, suggesting a possible role in intra-hand coordination. We conclude that the three-ball juggling task is be a promising example of a complex skill learning that can be studied with EEG to provide insights into the dynamics of neurobehavioral interactions between motor execution and perception.

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Poster

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Program #/Poster #: 662.21

Topic: H.10. Human Learning and Cognition

Title: Short reactivations over multiple days enhances motor memory retention and inter-manual transfer

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Abstract: Background: Practice is essential for skill acquisition, retention and generalization. When learning a new skill, performance improves rapidly, a stage referred to as early learning. These initial, early memories stabilize through consolidation, allowing the learner to return to subsequent practice sessions with superior skill levels. When previously consolidated memories are reactivated, they can experience further modification through reconsolidation. The influence of different reactivation lengths over multiple practice days on learning and on generalization in the form of inter-manual transfer of skill is unknown. Here, we addressed these questions. **Methods**: Participants (n = 64; age 18-35) practiced a modified star-drawing task virtually at home with their non-dominant left hand for 7 visits, with 14 days between visits 1 and 7. Participants performed 9 practice trials during Visit 1 and then 0, 1, 3, or 10 reactivation trials/visit (4 experimental groups) before testing retention (3 trials) and right-handed inter-manual transfer (3 trials) at Visit 7. A speed-accuracy tradeoff skill score was created using a separate validation group. Statistical analyses included two-way analyses of variance (Time x Group) of skill between visits 1 and 7 and between the end of Visit 7 and the inter-manual transfer test measurement. Change in skill between the first trial of Visit 1 with the left hand and the first trial of the inter-manual transfer test with the right hand was tested with a one-way between-groups analysis. **Results**: All groups learned between visits 1 and 7, with no between-group differences (Visit, $p < 0.001$; Group, $p = 0.790$; Interaction, $p = 0.378$). There were no between-group differences at Visit 7 ($p = 0.723$). Skill changed for all groups from Visit 7 to the transfer test (Visit, $p < 0.001$; Group, $p = 0.096$; Interaction, $p = 0.024$), with 3 Trials/Visit performing best at transfer ($p = 0.003$; all post-hoc pairwise comparisons between 3 Trials/Visit and all other groups were $p < 0.05$). The 3 Trials/Visit group demonstrated a greater change in skill between Visit 1 and transfer ($p = 0.001$; all post-hoc pairwise comparisons between 3 Trials/Visit and all other groups were $p < 0.01$). **Conclusions**: Long-term reactivation of a motor memory with as little as one trial leads to comparable retention levels as more trials, while there seems to be an optimal duration of reactivation trials to favor transfer to the non-trained hand. Investigation of optimal reactivation durations in the context of rehabilitation would be an important next step in optimizing rehabilitation treatments. Results may impact practice scheduling in sport, music, and rehabilitation.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.22

Topic: H.10. Human Learning and Cognition

Title: Theta-gamma neural signature for decoding individual finger movements during sequential skill learning

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Abstract: Background: Discrete finger movements have been decoded using invasive and noninvasive neuroimaging techniques. However, decoding individual finger movements within the context of highly coordinated sequential skill actions required for activities of daily living (e.g., typing) has been proven to be more challenging. Previous animal studies have proposed that the brain may represent action sequences as bursts of gamma oscillations nested into slower theta waves. We leveraged this knowledge to predict that theta/gamma interactions are critical to decoding of individual finger movements embedded within a continuous action sequence. Understanding this issue could contribute to increasing the degrees of freedom of brain-machine interfaces (BMIs) facilitating the rehabilitation of activities of daily living. **Methods**: We analyzed magnetoencephalography (MEG) recordings from 26 healthy volunteers while they learned a sequential typing task: 4 (index) - 1 (little) - 3 (middle) -2 (ring) - 4 (index)) with their non-dominant left hand for 12 minutes over 36 trials of practice (10 s each) interspersed with rest intervals (also 10 s each). We trained machine learning classifiers to decode each sequence item (keypress state corresponding to individual finger movement) using a combined theta (4 - 7 Hz) and gamma (31 - 59 Hz) band power feature and compared its performance against classifiers trained on features from narrow-band (theta, alpha (8 - 15 Hz), beta (16- 30 Hz), gamma) and broadband (1 - 59 Hz) neural oscillations. **Results:** The best performance was obtained with theta-gamma features in combination (mean weighted accuracy = 60.06%), which was significantly greater (1-tailed paired *t*-tests, $p < 0.05$ for all planned comparisons) than the rest of the features obtained from individual frequency bands (theta = 34.18%, alpha = 28.13%, beta = 28.97%, gamma = 34.65%) and broadband (36.47%) neural oscillatory activity. All features performed significantly higher (1-tail *t*-test, $p < 0.05$) than chance (p=0.25). The confusion matrix indicated higher dissimilarity between MEG keypress states for the little and index finger and most misclassifications occurred between the middle and ring finger states. **Conclusion:** We conclude that decoding individual finger movements in the context of continuous sequential actions is possible with non-invasive MEG. These results suggest the involvement of theta/gamma interactions in human sequential skill learning.

Disclosures: D. Dash: None. **F. Iwane:** None. **R.F. Salamanca-Giron:** None. **M. Bonstrop:** None. **E.R. Buch:** None. **L.G. Cohen:** None.

Poster

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Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.23

Topic: H.10. Human Learning and Cognition

Title: Anomalous slow-wave oscillatory brain activity predicts infrequent errors during skilled performance

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Abstract: Background: Many activities of daily living require skillful performance of motor sequences with exquisite spatial and temporal precision. In real life scenarios, errors performed during these types of skills (e.g. - the precise sequence of actions required when a pilot is manually landing a plane) can have catastrophic consequences. A previous fMRI study identified brain activity associated with performance of erroneous sequences. However, the millisecond-level brain activity preceding single erroneous components of skill sequences are not known. We predicted that application of machine learning algorithms to these data could enable us to decode errors from brain activity before they occur. **Methods**: We employed a well characterized procedural motor learning task while collecting behavioral and MEG data from 28 healthy volunteers who learned a sequential skill within a single training session. Participants were instructed to repeatedly type as many five-item keypress sequences (i.e. 4-1-3-2-4-) as possible on a keyboard. MEG was continuously recorded from 272 sensors (CTF275 MEG system) during 36 training trials. Each practice trial was 10s in duration and was interleaved with a 10s inter-trial rest interval. Keypresses deviating from the target were defined as erroneous. We investigated broadband MEG signal amplitude/power (0.5-40Hz) surrounding each error event. A one-class *isolation forest* machine learning technique was applied to determine the extent to which brain oscillatory activity surrounding erroneous keypresses differed from that surrounding correct keypresses. We also measured keypress transition times (KTTs). **Results**: Our analysis of MEG data found significantly anomalous delta-band (0.5-2Hz) amplitudes beginning 200ms prior to each erroneous keypress ($p < 0.05$, FDR corrected) and persisting for 1000ms afterwards. Higher frequency (3-40Hz) oscillatory brain activity had no significant predictive power related to error events. At a behavioral level, we found that KTTs preceding (474 ± 189 ms) and following (629 ± 259 ms; $p < 0.001$) an erroneous keypress were longer than those between consecutive correct keypresses (344 ± 129 ms; $p < 0.001$), consistent with a previous report by Gabitov et al.. **Conclusions:** Low frequency brain activity can predict errors in skill learning before they occur. These markers could be used to develop interventional brain computer-interface applications to prospectively arrest errors in the performance of highly skilled actions.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.24

Topic: H.10. Human Learning and Cognition

Title: Procedural Memory Learning in Patients with Long Covid

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Abstract: Background: Early in the Covid pandemic, reports started to emerge of patients with lingering symptoms following recovery from acute infection, often referred to as "Long Covid" (Miners, 2020; Nath, 2020; Troyer, 2020). Despite the high prevalence of neurological symptoms like brain fog and memory dysfunction in Long Covid (Davis, 2020), most research has relied on surveys or clinical tools typically used to assess declarative memory (Alemanno, 2021; Méndez 2021; Taquet, 2021). No prior studies to our knowledge have examined Long Covid patients' ability to learn and consolidate a procedural motor skill. **Methods:** We addressed this question in a group of 108 patients with Long Covid and 108 age- and sex-matched controls. Here, Long Covid was defined as self-reported persistent symptoms at least 3 months after onset of COVID-19 symptoms, with no fever for at least one week (World Health Organisation, 2021). Participants performed a well-characterized motor sequence typing task alternating 10-second practice with 10-second rest for 36 trials over 12 minutes (Bönstrup, 2019). The following day, performance was tested to evaluate overnight consolidation. The behavioral endpoint measure was correct sequence typing speed (Buch, 2021). Data were fitted to a model with 3-parameters (initial performance, maximum performance, learning rate). Simple reaction times (RT) were measured twice: at the beginning and the end of the experimental session. **Results:** On average, patients had experienced 50 weeks of symptoms at time of testing. Long Covid patients' typing speed was slower than healthy controls at the start of training ($p=0.00075$) and had not reached the same performance level by the end of training ($p=0.046$). Learning rates were statistically comparable across groups ($p=0.142$). Overnight consolidation was not statistically different between groups ($p=0.58$). There were no differences in any of the three measures between hospitalized patients and those who were not hospitalized. There were no sex differences in healthy or Long Covid groups. Pre-task RT was slower in Long Covid patients than in healthy controls (Covid 373±131ms, controls 317±43ms) but did not predict initial or final typing speed or learning rate in either group. Post-task RT was faster in both groups, marginally more so in patients (Covid 353±117ms, controls 314±51ms). **Conclusions:** Long Covid patients exhibited slower starting performance. While they learned the skill at about the same pace, they failed to reach the same typing speed as healthy controls by the end of the task. Reaction times did not predict initial typing speed, learning rate, or final typing speed.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 662.25

Topic: H.10. Human Learning and Cognition

Title: Kinematic synergies rapidly update to support procedural skill learning

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Abstract: Background: Performing complex motor behaviors likely relies on the refinement of coordinated effector kinematics, i.e. synergies. Performance improvements during motor learning have been linked to changing patterns of kinematic synergies. Here, we examined the change in specific kinematic synergies during early procedural skill learning, and we predicted that kinematic synergies rapidly transform following periods of rest to support early learning improvements. Methods: Twenty participants learned to type a numeric sequence (4-1-3-2-4) with the left, non-dominant hand as quickly and accurately as possible over 36 trials. Each trial consisted of 10s of practice followed by 10s of rest. Due to COVID restrictions, the study was run remotely with video supervision. A standardized keyboard and camera were delivered to each participant. Keypress performance was recorded using the keyboard with an online data collection platform. Movement was recorded with the camera, and kinematics were determined using markerless pose estimation software. Kinematic synergies were identified by non-linear dimensionality reduction of the movement data, using cluster totals of t-distributed stochastic neighbor embedding (t-SNE). Behavioral measures included: (a) early learning, the change in typing speed of correct keypresses/sec between trials 1 and 12; (b) microonline learning, the overall change in typing speed during practice periods; (c) microoffline learning, the overall change in typing speed between the end of each practice period and the beginning of the next; and, (d) the change in synergies associated with each keypress, measured by the mean absolute difference of y poses for each finger, sampled at each keypress, between trials 1 and 12. Results: Synergy count decreased progressively (36 trials, R^2 (adj.) = 0.89, p-value < 0.01). Synergy count reduction was greatest during early learning (trials 1-12, 10 $\pm$ 2). Synergy count reduction during early learning occurred largely during microoffline (rest) periods (microoffline, $p<0.01$). Over micro-offline periods, whole-hand synergy change was greatest at the ‘4’ and ‘1’ keypresses. Differences of y-pose for each finger at the ‘4’ and ‘1’ keypresses between trials 1 and 12 were significantly greater than other keypresses. Conclusions: Early procedural skill learning develops in parallel with a reduction of kinematic synergies. This synergy change

develops predominantly during rest intervals of practice (microoffline periods) driven predominantly by whole-hand pose transformations of the index and little finger.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Program #/Poster #: 662.26

Topic: H.10. Human Learning and Cognition

Support: ISCIII PI19/00298
ISCIII CP18/00038

Title: Posterior putamen activity is reduced during everyday-life habits in early Parkinson's disease

Authors: P. GUIDA[1], M. MICHIELS[1,2], M. H. MONJE[3], J. OBESO[1], ***I. OBESO**[4,5];
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Abstract: Habits allows us to perform well-practiced tasks with minimal effort, a function linked to sensorimotor territories of the cortico-basal ganglia loops. In Parkinson´s disease, initial loss of dopamine along the caudal sensorimotor putamen transfers to more rostral regions as disease progresses. Importantly, the role of sensorimotor putamen in habitual control could possibly explain some motor deficits part of everyday-life in PD such as arm swinging, walking and writing. The aim of this study was to investigate the influence of dopaminergic cell loss on motor and cognitive habits by means of behavioural and neural activations (fMRI) comparing newly diagnosed Parkinson's disease (PD) patients and healthy controls (HC). We developed an everyday-life motor task (handwriting) and a cognitive bias task (implicit bias measured by Go/noGo Associations Tasks, GNAT) to differentiate between habitual and goal-directed components. Patients were assessed behaviorally and inside a scanner while off medication. Behaviorally, handwriting performance in PD patients showed reduced automatism in writing kinematics in both habitual and goal-directed conditions compared to controls. However, in the cognitive habitual task, results reveal similar habitual responses and cognitive bias in both groups. In the motor task, neural signals ($p<0.05$, FPR-corrected) revealed enhanced activity in bilateral posterior putamen in controls in habitual conditions. In contrast, in goal-directed conditions, anterior putamen and caudate nucleus showed increased activity in controls. A similar pattern was observed in PD patients, however, showing a reduced posterior putamen

activity. The decrease of activity was stronger when the affected side coincided with the dominant hand. Cognitive habits showed that HC recruits posterior ipsilateral putamen in congruent/habitual trials and bilateral caudate nucleus for incongruent/goal-directed trials. In contrast, no habitual congruent activity was found in the striatum for PD patients while greater activity in the caudate for incongruent/goal-directed conditions. Our results suggest that everyday-life motor habits is executed with reduced automatic components in PD patients. However, at a functional level, both motor and cognitive habitual behaviour are accompanied by reduced posterior putamen recruitment in early PD. Our findings demonstrate a functional basal ganglia dysfunction that goes beyond habitual performance perhaps indicating a primary dysfunction of the posterior putamen associated with the highest dopaminergic loss.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Topic: H.10. Human Learning and Cognition

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Title: Resting-state GABA levels in sensorimotor and associated brain areas predict initial and long-term progress in a bimanual coordination task

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Abstract: The modulation of γ-aminobutyric Acid (GABA), the most important inhibitory neurotransmitter in the central nervous system, has been reported to play an important role in human learning. Thus far, several studies have addressed the associations between baseline GABA levels, as assessed by magnetic resonance spectroscopy (MRS), and motor learning ability. However, they mainly focused on the initial phase of skill acquisition. Here, we studied the role of resting-state GABA levels during both the early and late phases of motor skill learning under 2 different training conditions. A bimanual coordination task was used, consisting of 2 dials that controlled the direction and speed of a cursor on a PC screen. In each trial, participants were instructed to closely track the cursor on the screen by rotating both hands simultaneously. Fifty-one participants were randomly assigned to 2 groups and completed a

motor protocol consisting of 5 training sessions. In the visual feedback group (VFB, N=25), real-time augmented VFB was provided and participants could adjust their rotational movements online. In the no visual feedback group (NVFB, N=26), no real-time augmented VFB was provided during the trial but participants could see the trajectory of their movement after the completion of the trial. During each training session, Pre- and Post-tests were administered without feedback and task improvement was calculated as the performance difference between the Pre- and Post-test. Additionally, MRS assessed GABA levels were measured in 4 brain areas, i.e., the primary motor cortex (M1), the primary somatosensory cortex (S1), the dorsal-lateral prefrontal cortex (DLPFC), and the medial temporal cortex (MT/V5). Behaviorally, performance significantly improved during the initial sessions and reached a plateau during the last session. At the neural level, results from backward elimination regression showed that only S1 GABA levels were reserved as a predictor for initial ($F_{(1,23)} = 12.14$, $p < .002$) and long-term performance improvement ($F_{(1,23)} = 9.15$, $p < .006$) in the VFB group. Conversely, M1 and DLPFC GABA levels predicted initial ($F_{(2,23)} = 6.16$, $p < .007$) and long-term ($F_{(2,23)} = 4.96$, $p < .016$) performance improvement in the NVFB group. In conclusion, our findings suggest that behavioral progress under specific training conditions is associated with resting-state GABA levels in dedicated brain areas. Additionally, both initial and long-term progress were predicted by GABA levels in the same brain area. Together, these findings identify an important role for resting-state GABA levels in predicting learning ability for bimanual coordination tasks.

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Poster

662. Motor and Skill Learning: Human Behavior and Circuitry

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Topic: H.10. Human Learning and Cognition

Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development [1R01HD093624]
Clinical and Translational Science Award (CTSA) UL1TR002649

Title: Brain injury and problem solving skills in very preterm infants: a case series

Authors: *C. RHEE[1], C. BUTERA[1], B. SARGENT[1], R. MOLININI[2], J. WISNOWSKI[5], G. VORONA[3], D. BESSOM[6], M. SHALL[2], J. BURNSED[7], R. D. STEVENSON[8], S. BROWN[2], A. HARPER[4], K. HENDRICKS-MUÑOZ[9], S. DUSING[1,2,5];
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Abstract: Very preterm (VPT) birth increases the risk of brain injury (BI) and developmental delays. Infants move (motor skill) to learn (early problem solving). Early brain injury may contribute to delays in motor and problem solving skills. Here we explore the relationship between brain injury, motor skills, and problem solving for VPT infants monitored at short intervals. The ability to monitor at short intervals is crucial for use of assessments as evaluative measures of change in response to intervention.

Six VPT infants were analyzed. Neuroimaging, motor, and cognitive assessments were administered: 2-3 weeks before NICU discharge (V1), 15 weeks (V2), 30 weeks (V3), and 12 months (V4) post V1. Assessments included: Assessment of Problem Solving in Play (APSP; V2-V4), which is a 6-minute play-based assessment, Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III; cognitive [C] & motor [M] raw scores; V2-V4), General Movement Assessment (GMA; V1-V2), and Test of Infant Motor Performance (TIMP; V1-V2). Infants were randomized into a 15-week intervention encouraging parents to provide daily play-based learning activities (SPEEDI) or usual care groups. Non-sedated MRI scans were performed using a 3T Siemens Skyra scanner. MRI images were scored using the NINDS Neuroimaging Cerebral Palsy MRI Case Report Form. Descriptive statistics and Pearson correlation were computed.

At every visit, infants 1-3 had minimal BI, and infants 4-6 had no BI. All infants were born from 23-27 weeks gestation, and had a birth weight of 580g-1038g. All infants had a normal GMA at V2, suggesting low risk of cerebral palsy. The infants with BI scored lower on the TIMP at two consecutive timepoints, with 2 infants demonstrating a motor delay. All infants tested after V2 (n=5) improved their problem solving and BSID-III C and M. Infants 1 and 3 had positive correlations between APSP and BSID-III M (r's=.98, p's<.03) and C (r's>.96, p's<.03) across time. Infant 2 and Infant 4 had non-significant positive trends between APSP and BSID-III M ($r's$>.87, $p's$>.05) and C ($r's$>.81, $p's$>.08) across time. Infants 5 and 6 had too few visits to compute correlations.

We demonstrate positive relationships between APSP and BSID-III scores in VPT populations with BI, as seen previously in typically developing babies. These findings, combined with the ease of administration, training, and evaluative properties, support the use of the APSP to measure change in early problem solving at short time intervals. Research with larger sample sizes is needed to measure the effectiveness of APSP in short interval evaluations in VPT infants with BI.

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Poster

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Program #/Poster #: 662.29

Topic: H.10. Human Learning and Cognition

Support: JHU Science of Learning Grant

Title: Cross-sectional study of sensorimotor network functional connectivity during driving hazard perception in learner and experienced young drivers: insights into crash-risk difference

Authors: *T. J. CHIRLES[1], J. P. EHSANI[1], M. NEBEL[2,3], L. C. RICE[2], S. H. MOSTOFSKY[2,3], J. E. DESMOND[3];
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Abstract: Young drivers' crash risk is highest during the first year of independent driving, and this risk has been associated with the ability to detect and respond to driving hazards. Yet the neural underpinnings of driving hazard perception and how the cognitive process develops with experience are not well understood. Using a seed-based approach, we examined task-related differences in sensorimotor network (SMN) functional connectivity (FC) between young Experienced Drivers (ED) and Learner Drivers (LD) as they viewed near-crash events and routine driving. FMRI data were collected from 7 LD and 12 ED (16-21 years old) during our novel Driving Hazard Perception Task. LD had a learner's permit and <1,000 miles driving experience; ED had a driver's license for ≥ 2 years and drove >3,000 miles in the past year. The task consisted of 60 randomly-presented naturalistic driving videos that were either Event (evasive action needed to avoid driving hazard) or Non-Event (routine driving) 30s clips. Using a mixed effects model, we investigated regions where LD showed greater FC than ED during Events compared to Non-Events (LD>ED, Events>Non-Events) from the right postcentral gyrus (FSL Harvard-Oxford atlas). To determine directionality of the observed effects, we performed post-hoc tests for each group and condition separately. All results were thresholded at voxel-level $p<.005$, cluster-level FDR-corrected $p<.05$. During Event compared to Non-Event videos, LD showed greater FC between the right postcentral gyrus and nodes of the SMN (right lobules IV-VI, vermis VI, left postcentral gyrus) and ventral attention network (VAN) (right lobules VIII-IX), while ED showed decreased FC between these regions across the same conditions. Consistent with motor and skill learning theories, these results suggest that the cerebellum contributes to learning driving hazard perception. Specifically for LD, increased connectivity of cortical-cerebellar SMN regions with cerebellar VAN regions during hazardous "Event" scenarios suggests that this connectivity pattern may be crucial to developing internal models for learning evasive driving maneuvers, perhaps reflecting VAN importance in directing attention to salient stimuli. These observed neural adaptations associated with driving experience may serve as potential biomarkers to evaluate driving education programs and improve young driver safety.

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Poster

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Topic: H.10. Human Learning and Cognition

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HY-202000000002753

Title: Functional delineation of the human striatum related to reward processing in motor skill learning

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Abstract: The striatum is a major part of the basal ganglia to which the dopaminergic neurons are projected most, and it is important for motor control and reward processing. Thus, it is a locus of learning new motor skills based on reward feedback which reflects dopamine release. However, in human daily life, motor control and related reward processing are intertwined and reward feedback is often provided indirectly. To disentangle these roles of the striatum, we present an fMRI experiment of motor learning guided by continuous visual feedback of performance, which is explicitly provided throughout learning. We were able to delineate the anatomically defined striatum with high sensitivity and specificity using a parametric regressor encoding the learning performance. These selective striatal responses would support the idea that visual feedback functions as a reward signal, possibly reflecting dopamine release. Furthermore, we show that the anterior and posterior striatum have distinctive roles in reward processing and motor control, respectively. Taken together, our findings support the hypothesis that striatal BOLD responses represent a dynamic change in dopamine release that is intimately linked to motor learning performance.

Disclosures: S. Park: None. **J. Kim:** None. **S. Kim:** None.

Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.01

Topic: H.12. Aging and Development

Support: NIA Grant RF1AG039103
NIA Grant R21AG054197
NSF Grant 1633873

Title: Relationships between age, cortical thickness and cognitive performance

Authors: *M. DE CHASTELAINE[1], S. SROKOVA[2], M. HOU[3], A. KIDWAI[3], S. S. KAFAFI[3], M. L. RACENSTEIN[3], M. D. RUGG[3];
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Abstract: Both cross-sectional and longitudinal MRI studies have consistently reported declines in cortical thickness across the adult lifespan. Mean thickness has also been found to co-vary with cognitive performance; strikingly, whereas this relationship is positive in older adults, it has been reported to be negative in young adults. Here, we further investigated the relationships between age, cortical thickness and cognition across a large sample of healthy adults (n=375) that included three age groups: young (18-30 yrs), middle=aged (45-55 yrs) and older adults (63-76 yrs). Measures of cortical thickness were obtained using the semi-automated methods implemented in the Freesurfer package, and component scores on four cognitive constructs ('memory', 'speed', 'fluid processing' and 'crystallized IQ') were derived from a Principal Component Analysis of the scores obtained from a comprehensive neuropsychological test battery. Increased age was associated with a decline in both mean cortical thickness and scores on three of the cognitive constructs (memory, speed and fluid processing). Of importance, cortical thickness was negatively correlated with chronological age within both the young and older age groups. Mean thickness was negatively associated with memory scores in younger adults, whereas it was positively correlated with all four constructs in older adults; follow-up analyses revealed that these correlations were stronger for mean thickness of the right than the left hemisphere. Middle-aged adults did not show a relationship between cortical thickness and scores on any of the constructs. The findings are consistent with previous reports indicating that the direction of the association between cortical thickness and cognitive performance reverses between early and later adulthood, at least in the case of memory. Given the relatively narrow age range of the young and older groups, the group-wise findings are consistent with prior longitudinal studies that implicate aging, rather than cohort effects or selection bias, as the mechanism underpinning the association between age and cortical thinning across the lifespan. The neurobiological bases of the reversed relationships between cortical thickness and cognitive performance in young and older adults remain to be elucidated.

Disclosures: M. de Chastelaine: None. **S. Srokova:** None. **M. Hou:** None. **A. Kidwai:** None. **S.S. Kafafi:** None. **M.L. Racenstein:** None. **M.D. Rugg:** None.

Poster

663. Neural Mechanisms of Aging III

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.02

Topic: H.12. Aging and Development

Support: BYU Sayer Diabetes Fellowship

Title: A novel ketone-supplemented ketogenic diet improves recognition memory and hippocampal mitochondrial efficiency

Authors: *E. R. SAITO[1], C. E. WARREN[1], C. M. HANEGAN[1], J. LARSEN[1], J. D. DU RANDT[1], M. CANNON[1], R. J. CAMPBELL[1], J. Y. SAITO[1], C. M. KEMBERLING[1], G. S. MILLER[1], J. G. EDWARDS[2], B. T. BIKMAN[1];
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Abstract: Mitochondrial dysfunction and cognitive impairment are common symptoms in many neurologic and psychiatric disorders, as well as in nonpathological aging. Ketones have been suggested as therapeutic for their significance in epilepsy as well as other neurodegenerative diseases and mental health disorders such as Alzheimer's disease and major depressive disorder. However, their mechanistic effects on cognitive function in healthy individuals is less established. Here we explored the mitochondrial and performative outcomes of a novel eight-week ketone-supplemented ketogenic diet (KKD) on recognition memory and hippocampal mitochondrial bioenergetics in healthy adult male and female mice. Recognition memory and mitochondrial bioenergetics were both enhanced independent of changes in the expression of mitochondrial complexes and dynamics proteins. Together, these findings add to a growing body of evidence that suggest ketones and ketogenic diets are neuroprotective, and metabolically and cognitively relevant, even in healthy adults. They also suggest that ketogenic lifestyle changes may be effective strategies for protecting against cognitive decline associated with aging and disease.

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Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.03

Topic: H.12. Aging and Development

Title: Role of RIN3 in Alzheimer's Disease: Cellular and Molecular Mechanisms

Authors: *K. SUNG[1], R. SHEN[2], J. DING[2], C. WU[3];
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Abstract: BACKGROUND: Alzheimer's disease (AD) is a progressive disorder affecting learning and memory. Genetically, only 3-5% of AD cases are dominantly inherited while the vast majority is sporadic late onset of AD (LOAD). Results from genome-wide association study (GWAS) have uncovered ~40 risk factors associated with LOAD. RIN3, the Ras and Rab Interactor 3, a guanine nucleotide exchange factor (GEF) for the Rab5 small GTPase family, is implicated in both LOAD and sporadic early onset AD. The role of RIN3 contributing to AD pathogenesis is yet understood. **OBLECTIVE:** To define the cellular and molecular mechanisms and endocytic trafficking pathways by which RIN3 contributing to AD early cellular pathogenesis. **METHODS**: We use in vitro and in vivo models to investigate how increased expression of RIN3 alters endocytic trafficking and induces neurodegeneration. **RESULTS:** Our preliminary results have showed that RIN3 formed a complex with two additional AD risk factors: CD2AP and BIN1. Overexpression of RIN3 recruited CD2AP and BIN1 to Rab5 positive early endosomes. RIN3/CD2AP impaired APP trafficking and processing leading to accumulation of APP carboxyl terminal fragments; RIN3/BIN1 acted through GSK3beta to promote tau phosphorylation. A transgenic mouse model overexpressing RIN3 developed significant memory deficit similar to age-matched APP/PS1 mice; A RIN3 knockout mouse model did not show apparent learning and memory deficits. **CONCLUSION**: RIN3 is an important risk factor contributing to early cellular pathogenesis in AD. Increased expression of RIN3 impacts intracellular traffic of Rab5 early endosomes. The RIN3/CD2AP/BIN1 tripartite complex plays an important role in promoting amyloidogenic processing of APP and increasing tau phosphorylation. Investigation of cellular and molecular mechanisms underlying RIN3 function in AD will shed new insights into LOAD pathogenesis.

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Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.04

Topic: H.12. Aging and Development

Support: CIRM Training Fellowship

Title: Neuronal timescales across the lifespan: using human cortical organoids to study neurodevelopment and aging

Authors: *B. MARTIN-BURGOS[1], T. CHU[2], T. MCPHERSON[3], R. HAMMONDS[4], F. PUPPO[2], A. MUOTRI[5], B. VOYTEK[6];
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San Diego, San Diego, CA; [5]Pediatrics/Cellular Mol. Med., UCSD, La Jolla, CA; [6]Cognitive Sci., Univ. Of California, San Diego, La Jolla, CA

Abstract: In order to support complex cognition, neuronal circuits must integrate information across multiple temporal scales. The neuronal timescale—the duration over which the activity of a neuronal population typically persists—has the potential to explain how information might be maintained by spike trains over several orders of temporal magnitude. In recent work from our lab, we have demonstrated that neuronal timescales exhibit hierarchical spatial organization, mapping onto genetic and anatomical gradients in humans. Timescales are also functionally dynamic, and compress with age, highlighting the importance of studying how timescales change with development and aging. Despite recent progress in the study of timescales, little is known about the underlying circuits that give rise to them, nor to how they develop or change in aging. In order to understand the development of circuit and cellular level functional dynamics, we need invasive recordings sampled across the lifespan. Although long-term invasive data acquisition cannot be performed in humans, recently, cortical organoids generated from human induced pluripotent stem cells (hiPSCs) have emerged as a promising 3D model of human circuits. These cortical organoids allow us to study the long-term functional dynamics over development via electrophysiological recordings. In recent work from our lab, we used a novel spectral parameterization method to estimate timescales from multi-electrode array (MEA) recordings of human cortical organoids over development. We found that neuronal timescales follow an increasing and nonlinear developmental trajectory in neurotypical cortical organoids. To explore changes in timescales with aging, we used organoids with altered telomere length. Telomere shortening has been shown to relate to aging and life span across species. These organoids with shorter telomeres exhibited shorter, more compressed neuronal timescales, suggesting reduced memory capacity. This combined approach using novel timescale estimation methods on human cortical organoid models allows us to delineate how timescales emerge and change over healthy and disordered human lifespan compared to neurotypical development.

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Poster

663. Neural Mechanisms of Aging III

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Topic: H.12. Aging and Development

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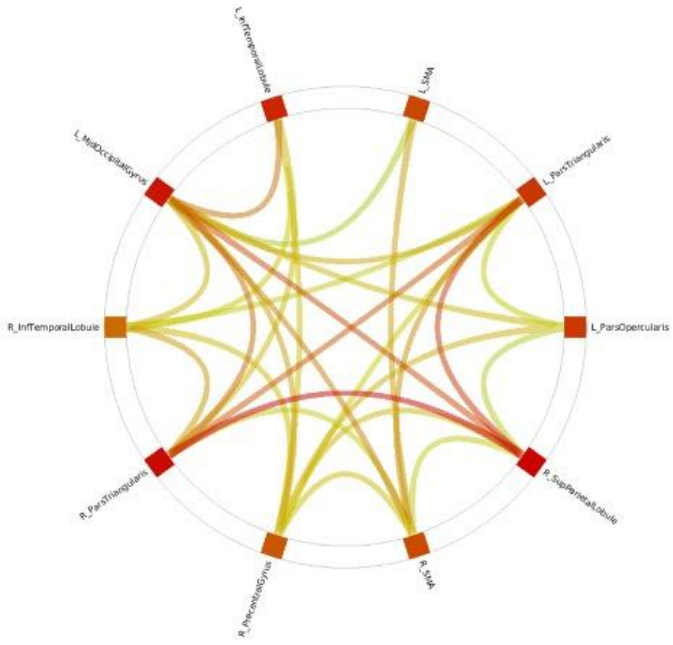
the McKnight Brain Research Foundation

Title: Task-based functional connectivity of the Useful Field of View (UFOV) fMRI task

Authors: *J. N. KRAFT[1], H. K. HAUSMAN[1], C. HARDCASTLE[1], A. ALBIZU[1], A. O'SHEA[1], N. D. EVANGELISTA[1], E. M. BOUTZOUKAS[1], E. J. VAN ETTEN[2], P. K. BHARADWAJ[2], S. G. SMITH[2], G. A. HISHAW[2], S. WU[1], S. DEKOSKY[1], M. MARSISKE[1], R. COHEN[1], G. E. ALEXANDER[2], E. PORGES[1], A. J. WOODS[1];
[1]Univ. of Florida, Gainesville, FL; [2]Univ. of Arizona, Tucson, AZ

Abstract: Declines in processing speed performance occur in aging and are a critical marker of functional independence in older adults. Numerous studies suggest that Useful Field of View (UFOV) training may ameliorate cognitive decline in older adults. Despite its efficacy, little is known about the neural correlates of this task. The current study investigated coherence of a functional connectivity network during UFOV task completion. 336 participants completed the UFOV task while undergoing task-based functional magnetic resonance imaging (fMRI). 10 *a priori* spherical regions of interest (ROIs) were created based on regions with the greatest peak BOLD activation patterns in the UFOV fMRI task, and regions that have been shown to significantly relate to UFOV fMRI task performance (Kraft et al., 2021). We used a weighted ROI-to-ROI connectivity analysis to model task-specific functional connectivity strength between *a priori* ROIs (Nieto-Castanon, 2020). We found that our UFOV network was functionally connected during task performance, and was significantly associated to UFOV fMRI task performance. Within-network connectivity of the UFOV network showed comparable or better predictive power in accounting for UFOV accuracy, compared to conventional resting state networks (frontoparietal control network, cingulo-opercular network, dorsal attention network, default mode network, visual, somatomotor and limbic networks). Finally, we demonstrate that the within-network connectivity of UFOV network accounted for scores on a measure of "near transfer", the Double Decision task, better than the aforementioned resting state networks. Our data elucidate functional connectivity patterns of a task central to an effective cognitive training paradigm shown to remediate age-related declines in cognition and reduce dementia incidence rate. These mechanistic data may assist in future targeted interventions, aiming to improve synchronicity within the UFOV network.



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Poster

663. Neural Mechanisms of Aging III

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Program #/Poster #: 663.06

Topic: H.12. Aging and Development

Support: CIHR Grant PJT-168974
NSERC Grant RGPIN-2018-04293

Title: Burnout and Resilience among Medical Professionals: An MRI Study

Authors: *A. ZAMANI[1], J. BURRELL[1], S. REYNOLDS[3], A. RAUSCHER[1], D. HODGES[3], K. CHRISTOFF[2];
[2]Psychology, [1]Univ. of British Columbia, Vancouver, BC, Canada; [3]Royal Columbian Hosp., New Westminster, BC, Canada

Abstract: Burnout is widespread yet remains poorly understood. Of particular concern is burnout in caretaking occupations, such as healthcare where it is associated with increased risk for patient and physician health. We used functional and structural MRI to examine burnout correlates and consequences in a population of intensive care physicians who regularly undergo prolonged week-long clinical duty shifts. We compared neural indices before and after a one-week long clinical shifts. Two scanning sessions per subject were acquired: one session a week prior to a clinical duty shift, and the other on the day immediately following the shift. At each scanning session we collected resting-state fMRI data and several other MRI sequences (T1, T2, T2-FLAIR, DWI, and ASL), as well as self-reported levels of burnout, resilience, and sleep characteristics. Results indicate changes in resting state functional connectivity (FC) following clinical week-long shifts, including FC between the subgenual anterior cingulate cortex and a number of cortical and subcortical structures, as well as between the insula and nucleus accumbens. These results add to previously reported relationships between medical burnout and alterations to insular brain activity and locus coerleus responsivity, as well as to resilience-dependent changes in subgenual anterior cingulate cortex functional connectivity following stress. Altogether, these results suggest a tentative link between burnout and alterations in brain systems supporting autonomic, visceral, and motivational processing.

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Poster

663. Neural Mechanisms of Aging III

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.07

Topic: H.12. Aging and Development

Support: NIH: AG046580
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Title: Brain acetylcholinesterase regulation and age-related changes in cognition

Authors: *A. KNIFFIN, C. BAVLEY, M. TARGUM, J. SEVERINO, J. FLOWERS, D. A. BANGASSER, M. E. WIMMER, V. V. PARIKH;
Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: Research has previously shown individual differences in cognitive aging; some individuals exhibit age-related cognitive decline while others do not. Cognitive decline in elderly populations serves as a risk factor for the development of Alzheimer's Disease and other forms of dementia. It is therefore imperative that we gain a comprehensive understanding of the neurochemistry driving age-related alterations to identify vulnerable populations and implement resilience mechanisms. The release of a brain chemical "acetylcholine (ACh)" from cholinergic neurons has been implicated in the neuromodulation of cognitive capacity. Our previous research

has shown that a reduction in ACh transmission in cortical networks produces age-related cognitive impairments. However, the mechanisms that regulate age-related changes in ACh transmission and individual differences in cognitive aging remain unknown. Acetylcholinesterase (AChE) is a hydrolytic enzyme which promotes ultra-fast cholinergic signaling within cortical networks. Here we sought to identify the role of synaptic (AChE-S) and readthrough (AChE-R) variants in AChE regulation and cognition in aging. Young and aged rats were trained in an operant attention task. Aged rats show higher performance variability with some animals exhibiting significant impairments while others performing at par with the young animals. Quantification of mRNA expression revealed a higher ratio of AChE-R/AChE-S variants in aged-performing rats in the prefrontal cortex (PFC). Moreover, a trend for an increase in the catalytic activity of AChE enzyme was also observed in this brain region. Because stress exposure has previously been shown to alter AChE expression and psychological stress is a risk factor for age-related cognitive decline, we also examined the effects of variable stress (VS) on AChE activity in adult rats. Our data show lower AChE activity in the detergent-soluble fraction isolated from the PFC of the VS-exposed rats suggesting adaptive stress-induced changes in cholinergic transmission. Moreover, the effect of VS on the expression of AChE variants interacted with sex, with females expressing lower S variant while males expressing higher S variant. Together, our results suggest disrupted AChE regulation presumably due to an imbalance between R and S variants may contribute to cognitive vulnerability in aging. Future research will seek to understand the mechanisms underlying dysregulation of AChE splice variants in stress and cognitive aging.

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Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.08

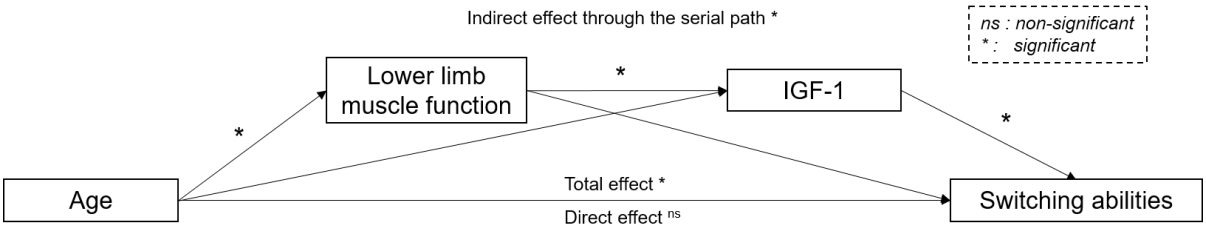
Topic: H.12. Aging and Development

Title: Igf-1 levels mediate the relationship between muscular aging and cognitive aging.

Authors: *A. LANGEARD[1], N. KAUSHAL[2], A. GAUTHIER[1], L. BHERER[3];
[1]COMETE U1075, Univ. de Caen Normandie, INSERM, Caen, France; [2]Indiana Univ., Indianapolis, IN; [3]Montreal Heart Inst. EPIC center, Univ. de Montréal, Montreal, QC, Canada

Abstract: An association between muscle and cognition in older adults has been reported, but underlying mechanisms and physiological factors remain poorly understood. It has previously been suggested that a possible mechanism through Insulin-like Growth Factor-1 (IGF-1) could explain this relationship between age-related muscle and cognitive declines. Participants (n=950) from the Midlife in the United States (MIDUS) study, aged between 34 and 84 years (55% women), were included. The Brief Test of Adult Cognition by Telephone evaluated cognitive

functions, including working memory, processing speed, and verbal fluency. Switching abilities were evaluated through the Stop & Go Switch Task (switching cost). Chair stands were used to measure lower limb muscle function, and grip strength was used to measure upper limb muscle function. Blood samples were collected from each participant to measure biomarkers, including fasting IGF-1 levels. Serial mediation analyses were used to determine if the effect of age on the different cognitive outcomes was mediated by the different muscle outcomes' and their association with biomarkers levels. The age effect on switching scores was significantly mediated by the chair stand score associated with IGF-1 blood levels. These mediators accounted for 36% of the total effects of age on cognitive decline. When accounting for these mediators, the relationship between age and switching was no longer significant, supporting a full mediation hypothesis. These findings were not as consistent for other biomarkers, upper limb function and other cognitive functions. Age-related muscular decline, particularly at the lower limb level, may explain some of the cognitive declines observed with aging, particularly in executive function. This relationship is related to a decline in IGF-1 blood levels. These findings may explain the association between sarcopenia (age-related loss of skeletal muscle mass) and cognitive decline but also highlight the cognitive interest of exercise training aiming at increasing muscle mass and function.



Disclosures: **A. Langeard:** None. **N. Kaushal:** None. **A. Gauthier:** None. **L. Bherer:** None.

Poster

663. Neural Mechanisms of Aging III

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Topic: H.12. Aging and Development

Support: NIH Grant R21 AG048431
NSERC Discovery PGPIN - 2016-05343

Title: The role of intrinsic functional connectivity in cognitive reserve: Evidence from bilingualism in older adults

Authors: *N. KHAN[1], W. D. STEVENS[1], J. A. E. ANDERSON[2], C. L. GRADY[3], E. BIALYSTOK[1];
[1]Psychology, York Univ., Toronto, ON, Canada; [2]Cognitive Sci., Carleton, Ottawa, ON, Canada; [3]Rotman Res. Inst. at Baycrest Hosp., Toronto, ON, Canada

Abstract: A rapidly aging population across the globe has brought age-related cognitive decline to the forefront of priorities of health policymakers. Cognitive Reserve (CR) refers to the preservation of cognitive function in the face of age- or disease-related neuroanatomical decline. Older adults with high CR maintain cognitive performance in the face of age-related neuroanatomical decline. Several lifestyle factors, including lifelong bilingualism, have been identified that contribute to CR. However, there remains a need to better understand the largely unknown neural mechanisms of CR. Here, we tested the hypothesis that intrinsic functional connectivity is a neural mechanism of CR by comparing the structural and functional integrity of large-scale brain networks in cognitively matched bilingual and monolingual older adults. We predicted that bilingual older adults would show reduced structural integrity but preserved functional integrity compared to monolinguals. Using voxel-based morphometry, we observed that bilingual older adults had reduced gray matter integrity in the posterior cingulate cortex, a core region of the default mode network, but preserved functional segregation between the frontoparietal control network and default mode network. Next, using graph theoretical analysis, we tested for group differences in the betweenness centrality of the posterior middle frontal gyrus - a node in the frontoparietal control network that has been identified as playing a key role as a connector between networks. Bilinguals showed preserved betweenness centrality of the posterior middle frontal gyrus, providing further evidence of maintained functional integrity in bilinguals. Finally, we collapsed across groups and tested the relationship between these brain measures and second language proficiency, a continuous variable across all older adults. Second language proficiency was negatively associated with structural integrity and positively associated with functional integrity measures. These findings confirm that lifelong bilingualism is a lifestyle factor contributing to CR and demonstrate that experience-dependent maintenance of the frontoparietal control network is a neural mechanism of CR.

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Poster

663. Neural Mechanisms of Aging III

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.10

Topic: H.12. Aging and Development

Title: Differences in Resting State Functional Connectivity in Early and Late Post-menopausal Women

Authors: *A. TESTO[1], J. A. MAKAREWICZ[2], E. MCGEE[3], J. A. DUMAS[4];
[1]Univ. of Vermont Neurosci. Grad. Program, Burlington, VT; [3]Dept. of Obstetrics, Gynecology, and Reproductive Sci., [2]Univ. of Vermont, Burlington, VT; [4]Psychiatry, Univ. of Vermont Larner Col. of Med., Burlington, VT

Abstract: Title: Differences in Resting State Functional Connectivity in Early and Late Post-menopausal WomenAuthors: Abigail Testo[1], Jenna Makarewicz[1], Elizabeth McGee[2], Julie Dumas[11]Department of Psychiatry[2]Department of Obstetrics, Gynecology, and Reproductive SciencesUniversity of Vermont, Burlington, VT 05401
Background: Functional connectivity characterizes functional connections within the brain and refers to the similarity of signals arising from anatomically distinct brain regions. Previous studies have found that estrogens play a role in functional connectivity in the brain, however, little research has been done regarding how functional connectivity changes following the decline in estrogen levels that occurs following the menopausal transition. The purpose of this study is to examine the potential differences in functional connectivity that may exist between women who have recently completed the menopausal transition according to the Stages of Reproductive Aging Workshop (STRAW) +10 and those who have been postmenopausal for greater than 5 years.
Methods: Structural and BOLD resting state MRI scans of 88 cognitively healthy postmenopausal women were collected and used to generate connectivity values in CONN toolbox version 20.b, an SPM-based software. Of those women, 55 were STRAW +1 group (mean age = 55.67, SD=2.51) and 33 were STRAW +2 group (mean age 57.42, SD=2.18) and connectivity values between groups were compared. Regions of interest included brain regions within the prefrontal cortex due to the presence of estrogen receptors found there.
Results: Women with a STRAW score of +1 had significantly greater connectivity at rest between the frontal parietal network when the starting seed was set to left lateral prefrontal cortex (coordinates -43, 33, 28) and a cluster located at coordinates -02, -52, -14. The cluster survived both FWE (p<.001) and FDR (p=.005) corrections. Approximately one third of the cluster was located within the Cerebellum.
Conclusions: These results demonstrate the influence of menopausal stage on functional connectivity even among women within a small age range. They have implications for understanding how the functioning of the brain changes for women after menopause that may eventually lead to changes in cognition and behavior in older ages

Disclosures: **A. Testo:** None. **J.A. Makarewicz:** None. **E. McGee:** None. **J.A. Dumas:** None.

Poster

663. Neural Mechanisms of Aging III

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Program #/Poster #: 663.11

Topic: H.12. Aging and Development

Support: CIHR Grant awarded to M. N. Rajah

Title: Alterations in white matter microstructure associated with menopause

Authors: *R. LISSAMAN[1,2], S. RAJAGOPAL[2], H. AZIZI[2], L. KHAYYAT[2], R. YOUNG[2], A. CRESTOL[2], S. PASVANIS[2], M. N. RAJAH[1,2];

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Abstract: Estrogens have widespread effects throughout the brain. During menopause, estrogen levels – notably 17β estradiol – markedly decline. This decline has been linked to changes in brain function and cognition in humans, and with increased levels of Alzheimer's disease (AD) pathology in animal models. However, it is not well-established whether menopause is also associated with alterations in white matter, which is critical for human brain health and is often damaged in neurodegenerative conditions such as AD. To address this, we used diffusion tensor imaging to investigate the effect of menopause on white matter microstructure. The sample comprised female participants (current n = 93; mean age = 44.8 years; age range = 21-64.5 years) recruited from the Montréal area. All participants underwent an in-person neuropsychological evaluation and an MRI scanning session. Menopausal stage (pre- & post-menopause) was ascertained using STRAW-10 guidelines. We used both univariate and multivariate statistical analyses to examine differences in fractional anisotropy (FA) and mean diffusivity (MD) – two measure of white matter microstructure – among pre- (n = 52) and post-menopausal (n = 41) females. Univariate analyses identified statistically significant differences in FA, with pre-menopausal females showing higher FA throughout much of the white matter. After adjusting for age, however, no differences remained. To examine regional differences, analyses were re-run with age and global values included as covariates. These analyses revealed that FA was higher in the superior longitudinal fasciculus and internal capsule among pre-menopausal females. No age-adjusted global or regional differences were observed for MD. A preliminary multivariate partial least squares analysis of FA identified a single significant latent variable, reflecting a menopause x age x education interaction. Specifically, across numerous white matter tracts, FA was positively associated with age in pre-menopausal females and with years of education in post-menopausal females. Together, these findings provide initial evidence that the transition from pre- to post-menopause is associated with alterations in white matter microstructure, and highlight how certain factors may be related to microstructure at specific stages of menopause.

Disclosures: R. Lissaman: None. **S. Rajagopal:** None. **H. Azizi:** None. **L. Khayyat:** None. **R. Young:** None. **A. Crestol:** None. **S. Pasvanis:** None. **M.N. Rajah:** None.

Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.12

Topic: H.12. Aging and Development

Support: NIH R01HL102457

Title: Arterial Stiffness Mediates the Association between Aging and Hippocampal Diffusivity

Authors: *J. WON, T. TOMOTO, T. TARUMI, R. ZHANG;
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Abstract: Objective and Rationale: Aging is associated with changes in hippocampal microstructural integrity. The objective of the present study was to investigate if age-related differences in hippocampal microstructural integrity assessed with diffusion tensor imaging (DTI) are mediated by central arterial stiffness - a hallmark of vascular aging. **Methods**: One hundred forty healthy adults [33 younger adults (30 ± 5 years), 42 middle-aged adults (50 ± 5 years), and 62 older adults (67 ± 5 years)] underwent measurements of carotid-femoral pulse wave velocity (cfPWV) and local arterial stiffness (carotid β-stiffness index) at the common carotid artery using tonometory and ultrasonography. Bilateral hippocampal fractional anisotropy (FA) and mean diffusivity (MD) was measured using DTI. Amygdala diffusion was assessed to test the specificity of the associations. Linear regression was used to examine the associations between aging, arterial stiffness, hippocampal FA, and MD after controlling for sex, education years, systolic blood pressure, and use of blood pressure medication. Mediation analysis was performed to examine the relationship between aging, hippocampal microstructural integrity, and arterial stiffness. **Results**: Mean cfPWV (m/s) of each age group was 6.0 ± 0.7 for younger, 7.2 ± 1.3 for middle-aged, and 8.6 ± 1.5 for older adults. Mean carotid β-stiffness index (a.u.) of each age group was 5.6 ± 1.1 m/s for younger, 7.4 ± 1.4 m/s for middle-aged, and 9.8 ± 2.1 for older adults. Age was also associated with higher cfPWV ($\beta = 0.667$, $p < 0.0001$) and higher carotid β-stiffness index ($\beta = 0.740$, $p < 0.0001$). Age was associated with lower hippocampal FA ($\beta = -0.258$, $p < 0.0001$), greater hippocampal MD ($\beta = 0.295$, $p < 0.0001$), and lower hippocampal volume ($\beta = -0.251$, $p < 0.001$). No significant associations were found between age and amygdala FA and MD ($p \geq 0.142$). Higher cfPWV ($\beta = 0.246$, $p = 0.026$) and carotid β-stiffness index ($\beta = 0.304$, $p = 0.008$) were associated with greater hippocampal MD. The associations between age and hippocampal MD were mediated via cfPWV (95% CI 0.0001, 0.011) and carotid β-stiffness index (95% CI 0.0002, 0.014), respectively. **Conclusion**: Central arterial stiffening may play an important role in age-related hippocampal microstructural integrity.

Disclosures: J. Won: None. **T. Tomoto:** None. **T. Tarumi:** None. **R. Zhang:** None.

Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.13

Title: WITHDRAWN

Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.14

Topic: H.12. Aging and Development

Support: NIH Grant AG072328

Title: Locus coeruleus structure and catecholamine synthesis capacity interact to predict cognitive functions in aging

Authors: *H.-Y. CHEN[1], J. H. PARENT[1], C. J. CIAMPA[1], M. J. DAHL[2], D. HAEMMERER[3], A. MAASS[4], J. R. WINER[6], B. INGLIS[7], W. J. JAGUST[8], M. J. BETTS[5], A. S. BERRY[1]; [1]Psychology, Brandeis Univ., Waltham, MA; [2]Max Planck Inst., Berlin, Germany; [3]Inst. of Cognitive Neurol. and Dementia Res., Universitätsklinikum Magdeburg, Magdeburg, Germany; [4]Inst. of Cognitive Neurol. and Dementia Res., [5]German Ctr. For Neurodegenerative Dis. (DZNE), Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany; [6]Neurol., Stanford Univ., Stanford, CA; [8]Publ. Hlth. and Neurosci., [7]Univ. of California, Berkeley, Berkeley, CA

Abstract: The locus coeruleus (LC) modulates a wide range of cognitive functions including episodic memory and executive function, and is implicated in the early pathophysiology of Alzheimer's disease (AD). Novel structural magnetic resonance imaging (MRI) approaches detect meaningful individual differences in LC structural integrity that correlate with cognitive performance and AD pathology burden in aging and across the AD spectrum. However, it is unclear to what extent MRI measures of the LC reflect the underlying neurochemical synthesis capacity within the LC. We present preliminary data addressing this question. Nineteen cognitively normal adults (age range 20-84 years) underwent 3T MRI imaging of the LC (magnetization transfer contrast) and [^{18}F]Fluoro-m-tyrosine ([^{18}F]FMT) PET, which measures catecholamine (norepinephrine/dopamine) synthesis capacity. We found no direct association between LC MRI and [^{18}F]FMT PET measures for the analyses using MRI-defined LC regions of interest comprising the whole LC, or rostral and caudal subregions (partialing out chronological age). Next, we tested the extent to which LC MRI and [^{18}F]FMT PET are associated with cognitive performance. We applied a partial least square correlation (PLSC) analysis to isolate a single latent variable (p = 0.02) that optimally expresses the multivariate association between participants' LC brain measures and cognitive performance (r = 0.78; [95% confidence interval: 0.35 0.87]). Specifically, the rostral LC MRI and the rostral LC MRI * [^{18}F]FMT PET interaction were the primary contributors to this latent variable, which was further associated with neuropsychological tests related to episodic memory (e.g., visual reproduction [r = 0.48, p = 0.04]) and execution function (e.g., trail making B [r = -0.53, p = 0.03] and backward digit span [r = 0.67, p = 0.002]). While preliminary, these data suggest that LC MRI and [18F]FMT PET measures may provide unique information and their conjoint use may improve sensitivity in small samples.

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Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

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Program #/Poster #: 663.15

Topic: H.12. Aging and Development

Support: NIH Grant R56AG060052

Title: Increasing attentional control demands in working memory training engenders cognitive plasticity through changes in left fronto-parietal brain structures

Authors: *C. BASAK[1], P. A. SKOLASINSKA[1], E. T. SMITH[1], S. QIN[2], G. H. SHERARD[1], D. C. PARK[1], P. FISHWICK[1];
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Abstract: The primary aim of the current Phase I clinical trial (**NCT03988829)** was to determine whether working memory training that requires higher cognitive control, compared to a similar training that requires lower cognitive control, results in greater cognitive enhancements in normal aging. If so, complimentary changes in brain structure would implicate cognitive *plasticity*, not just cognitive *flexibility*. Fifty-one older adults were randomized into three training arms, where cognitive control demands during working memory updating were systematically increased using game-based simulations. Given COVID interrupting the study, data was primarily collected on arms that used a in-house *Birdwatch* game, where either predictable (Low-Control) or unpredictable (High-Control) shifts of cued-attention were needed. Completed dataset, including neuroimaging and cognitive metrics, at both pre- and post-training assessment sessions were collected on 30 older participants, with game learning metrics on 28 participants. High-Control Training yielded significant gains in overall cognition when compared to Low-Control Training (*Cohen's d* for the ArmxSession interaction =1.21). These gains favoring High-Control Training were mainly observed for executive function and processing speed constructs. However, no differential improvements were observed in either game learning or psychosocial abilities. These differential cognitive gains were accompanied by significant increases in left lateral parietal gray matter volume (*Cohen's d* =0.87) and its cortical thickness (*Cohen's d* =0.88), and increases in left lateral frontal gray matter volume (*Cohen's d* =0.72) for High-Control, compared to Low-Control, Training. Accompanying increases in white matter integrity that connects left frontal and left parietal brain regions were also observed. Structure-Cognition relationships suggests that these increases in left fronto-parietal gray matter volumes and white matter integrity are related to improved cognition, but only for High-Control training group. Our results suggests that high control cognitive training induces cognitive plasticity, not mere flexibility.

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Poster

663. Neural Mechanisms of Aging III

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Program #/Poster #: 663.16

Topic: H.12. Aging and Development

Support: NIH/NIA Grant 1RF1AG062831
NIH/NIA Grant 2RF1AG043640

Title: Immune proteins may contribute to aberrant microglia phagocytosis leading to synapse loss and associated cognitive impairment in the aging monkey

Authors: *S. A. DEVRIES, B. CONNER, M. MEDALLA, F. MORTAZAVI, D. L. ROSENE; Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Normal aging humans are free of Alzheimer's Disease (AD) but nevertheless suffer from age-related cognitive impairment. Studies of normal human aging, however, are often confounded by early-state AD. The aging monkey has provided considerable insight into normal human aging since they do not develop AD but exhibit cognitive impairment similar to humans. Research in both humans and monkeys has shown that neurons are not lost in normal aging. Instead, there is myelin damage, synapse loss, and dendritic atrophy, especially in the dorsolateral prefrontal cortex (dlPFC), an area critical to cognition. While it is possible that synapse loss is secondary to myelin damage, another possibility is that synapses are aberrantly eliminated by microglia, the resident immune cell of the central nervous system. Microglia-mediated phagocytosis is modulated by immune "eat me" and "don't eat me" signaling proteins. "Eat me" signals, including complement components, appear to direct microglia phagocytosis in an activity-dependent manner, so that less active synapses are eliminated. Previous research has focused on "eat me" signaling but has largely ignored the "don't eat me" signals. This study aimed to investigate the balance between the "eat me" signal, complement component C1q and the "don't eat me" signal CD47 relative to age-related synapse loss in the dlPFC. To do this, tissue available from 32 cognitively tested male and female rhesus monkeys (7 to 30 years of age) was selected. Cognitive testing for learning and memory included the delayed-nonmatch to sample (DNMS) and delayed recognition span (DRST) tasks. Scores from these tasks are used to calculate a cognitive impairment index (CII) that confirmed age-related cognitive impairment. To investigate colocalization of C1q and CD47 with postsynaptic densities in the dlPFC, quadruple label immunofluorescence staining for postsynaptic density 95 (PSD95), neuronal marker NeuN, C1q, and CD47 was performed. To determine if microglia phagocytosis correlates with age-related changes in these immune signals, immunohistochemistry was performed using LN-3 and Galectin-3 to quantify the density of activated and phagocytic microglia, respectively. Overall, results show elevated C1q along with diminished CD47 levels with age as well as increased phagocytic microglia. These findings suggest that with age, active and phagocytic microglia increase in the dlPFC and receive increased "eat me" signals from C1q along with reduced "don't eat me" signaling from CD47, suggesting a possible mechanism for age-related synapse loss and associated cognitive impairment. Supported by NIH/NIA grants 1RF1AG062831 and 2RF1AG043640.

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Poster

663. Neural Mechanisms of Aging III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 663.17

Topic: H.12. Aging and Development

Title: Hemodynamic response variability and its relationship to the BOLD signal in younger and older adults

Authors: *M. TAYLOR[1], M. P. TURNER[4], K. WEST[4], D. H. ABDELKARIM[5], Y. ZHAO[2], B. P. RYPMA[3];
[1]Univ. of Texas At Dallas, Grand Prairie, TX; [2]Univ. of Texas At Dallas, Addison, TX; [3]Behavioral & Brain Sci., Univ. of Texas At Dallas, Dallas, TX; [4]The Univ. of Texas at Dallas, Richardson, TX; [5]UT Dallas, Allen, TX

Abstract: The blood-oxygen-level-dependent (BOLD) signal yields a characteristic "hemodynamic response function" (HRF) that reflects a combination of blood-flow and oxygen-hyperperfusion changes that follow neural activity. In healthy aging, multiple components of the HRF (e.g., rise time, peak amplitude, and fall time) are susceptible to the mediating effects of age-related cerebrovascular alterations and underlying processes. Additionally, previous studies from our lab have demonstrated that neurovascular coupling differences adults are mirrored in HRF differences. These differences include an increased time-to-peak and a decreased peak amplitude of the HRF. To further explore these phenomena, the current study utilized the publicly available Cambridge Center for Aging and Neuroscience (CamCAN) dataset to estimate variability in HRF amplitude in a visual-auditory task in 80 younger (18-30 years old; 44 Female/36 Male) and 212 older adults (54-74 years old; 100 Female/112 Male). Variance of BOLD signal amplitude was calculated for 12 time points past onset of a flickering checkerboard paired with an auditory tone across 40 trials. An F-test was performed to test equality of variances between the young and old groups and between ROIs for both groups. We found that older adults had increased variance in the occipital region compared to younger adults ($F = 0.23$, $p = .021$). Additionally, younger adults had decreased variance in the temporal compared to the occipital region ($F = 4.32$, $p = .023$; $F = 7.96$, $p = .002$; $F = 4.32$, $p = .023$). These results demonstrate that HRF shape is altered in the aging process, possibly resulting from changes to the underlying neurovascular coupling processes.

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Poster

663. Neural Mechanisms of Aging III

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Program #/Poster #: 663.18

Topic: H.12. Aging and Development

Support: NIMH K01MH099232 (AJH)
Tan Ean Kiam Postgraduate Scholarship (RC)

Title: The genetic and cellular bases of white matter microstructure in late life

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Abstract: Microstructural architecture of white matter (WM) affects information flow across local circuits and large-scale networks. WM tract integrity is linked to variability in cognitive traits across healthy, neurodegenerative and/or psychiatric populations (Fields, 2008). Neuroanatomical components underlying diffusivity measures have largely been studied in animal models. Axial diffusivity has been shown to track axonal integrity (Sun et al, 2006), while radial diffusivity changes likely reflect damage to the myelin sheath (Song et al, 2005). Despite the importance of anatomical connections for brain function and behavior, less is known about heritable factors underlying WM microstructure in humans, particularly the extent to which genetic contributions may bias tract integrity across the lifespan. While differential aging trajectories are evident across WM tracts (Chong et al, 2012), the role of associated cell types is less systematically investigated. We clarified genetic factors underlying individual differences in diffusivity measures using a population-based cohort (N=29,862) from the UK Biobank. Using diffusion measurements calculated by tract based spatial statistics derived from 432 imaging phenotypes (Miller et al, 2016), we performed 1) heritability analyses on 10 diffusivity measures across 48 WM tracts, 2) estimated shared/independent genetic influences amongst tract classes, and 3) used polygenic cell deconvolution to establish cellular associates of age-related declines in WM. Individual variability in WM tracts is under genetic control (mean h^2=.28), with ~98.5% of indices displaying significant SNP heritability ($p<.05$). Genetic correlation analyses revealed increased correlations for pairings *within* WM tract families than *between* families ($p<.05$) with significant age decline on FA ($p<.01$) and conversely, increased MD measures across WM tracts with increasing age ($p<.01$). Cell-type analyses showed oligodendrocyte enrichment and WM markers of aging, where tracts with higher enrichment for oligodendrocyte linked genes exhibited greater age related decline in FA ($r=-.40$, $p<.005$) and conversely, greater age associated increase in MD ($r=.31$, $p<.032$). Tract pairings within classes exhibited greater genetic covariance relative to those between classes, suggesting shared genetic influences *within* WM tract classes. Oligodendrocyte-linked genes accounted for a significant proportion of heritable variance in tracts marked by age related decline in FA and MD, with association tracts exhibiting the greater effects, highlighting a role for oligodendrocyte related transcripts in late life structural brain changes.

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Poster

663. Neural Mechanisms of Aging III

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Program #/Poster #: 663.19

Topic: H.12. Aging and Development

Support: R01GM134363

Title: Age-related changes in alpha and mu oscillation amplitude and waveform asymmetry

Authors: *A. BENDER[1], B. VOYTEK[2], N. SCHAWORONKOW[3];
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Abstract: Neural rhythms in the human brain are most prominent in the alpha band (8-13 Hz). While EEG alpha rhythms in occipital cortex and mu rhythms in sensorimotor cortex occur at similar frequencies, alpha and mu are thought to have different neural generators. Because neural rhythms are commonly nonsinusoidal, and the nonsinusoidal features have been shown to reflect underlying physiological and pathophysiological characteristics (Cole et al., 2017), quantifying the differences in waveform shape between alpha and mu rhythms may inform understanding of the physiological distinctions between these rhythms. In this study, we quantified changes in waveform shape of both alpha and mu rhythms across adulthood. We analyzed an open dataset of cross-sectional resting-state EEG from 228 participants, comprised of a younger (n=154, range 20-35 years, 45 females) and an older group (n=74, range 59-77 years, 37 female) of adults (Babayan et al., 2019). We used spatio-spectral decomposition to extract rhythmic components with enhanced signal-to-noise ratio in the alpha/mu band. The spatial patterns for each of these components, which represent how each rhythmic source maps onto the electrodes, were matched to alpha and mu template patterns to identify whether each component likely represented an alpha or mu rhythm. We then used the Python bycycle waveform analysis package (Cole & Voytek, 2019) to quantify waveform shape features on a cycle-by-cycle basis. Using this approach, we found that alpha ($t(80)=0.43$, Cohen's $d=-0.145$) and mu ($t(71)=-0.01$, $d=0.003$) center frequency decreases only minimally across adulthood, while average cycle-by-cycle amplitude increases for both alpha ($t(80)=2.62$, $d=0.731$) and mu ($t(71)=5.15$, $d=1.515$) waveforms. Finally, we demonstrated that alpha ($t(80)=4.38$, $d=1.109$) and mu ($t(71)=6.39$, $d=1.503$) waveforms become significantly more asymmetrical with age. These results suggest that, while much of the previous literature on rhythms has focused on center frequency and amplitude, nonsinusoidalities are the most prominent change in waveform shape across adulthood and important features of rhythms that warrant further study.

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Poster

663. Neural Mechanisms of Aging III

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Program #/Poster #: 663.20

Topic: H.12. Aging and Development

Support: BMBF Grant 01GQ1421B

Title: Aerobic exercise is associated with region-specific changes in white matter volume, and tensor- and fixel-based diffusion measures of white matter integrity in healthy older adults

Authors: *S. E. POLK[1,3], M. M. KLEEMEYER[1], N. C. BODAMMER[1], S. KÜHN[2], U. LINDENBERGER[1,4], S. DÜZEL[1], E. WENGER[1];
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Abstract: Deterioration of white matter (WM) integrity and cognition commonly seen in older adults may be ameliorated through modifiable lifestyle factors such as aerobic exercise. WM integrity can be estimated with a number of magnetic resonance imaging (MRI)-based parameters, including WM volume (WMV) and the diffusion tensor-derived parameters of fractional anisotropy (FA) and mean diffusivity (MD). Notably, the tensor model can only adequately model the diffusion of water molecules in tissue if there is a single main diffusion orientation (interpreted as one fiber orientation) present in a voxel, leading to a lack of validity of these tensor-derived measures within voxels with crossing white matter fibers (i.e., multiple fibers with different orientations). In an attempt to overcome this limitation, more complex models describing diffusion in multiple orientations per voxel have been developed (e.g., constrained spherical deconvolution), which make use of "fixels," or specific fiber bundles within a voxel. Using such fixel-based techniques, measures such as fiber density (FD) and cross-section (FC), as well as the product of these parameters, fiber density and cross-section (FDC), can be estimated in multiple directions within each voxel. We measured changes in WM integrity, estimated using WMV and the tensor-derived metrics, as well as the newer fixel-based parameters, following a six-month at-home intervention in 61 adults, aged 63 to 76 years, who were assigned to either a moderate aerobic exercise group or an active control group. Correlations among changes in indices of WM integrity, cardiovascular fitness, and performance on the Digit Symbol Substitution task (DSST), a marker of perceptual speed, were also investigated. We observed maintenance of WMV in the corpus callosum of the exercise group, corroborating previous research, as well as positive change-change correlations between WMV and fitness, and between WMV and DSST score. Surprisingly, exercisers showed more negative changes in FA and more positive changes in MD than controls in the corpus callosum, posterior corona radiata, and superior longitudinal fasciculus, as well as more negative changes in FD and FDC in the prefrontal cortex. Interestingly, these negative changes in FD and FDC in superficial white matter were found to be inversely correlated to changes in both fitness and DSST score. In conclusion, these results support previous findings regarding the effects of aerobic exercise on

WMV, but raise questions regarding the physiological interpretation of both tensor- and fixel-based parameters of WM integrity.

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Poster

663. Neural Mechanisms of Aging III

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Program #/Poster #: 663.21

Topic: H.12. Aging and Development

Support: NIA IRP

Title: Probing the role of histone acetylation in age-related cognitive decline cognitive decline

Authors: *L. WANG[1], R. A. MCDEVITT[2], N. YANG[1], C. SHI[1], J. OCCEAN[1], P. SEN[1]; [1]NIH Hlth. Sci. Campus, Baltimore, MD; [2]Comparative Med., NIA, Baltimore, MD

Abstract: Aging is accompanied by cognitive deficits, with the occurrence of broad and extensive changes in chromatin. Previous reports suggest that chromatin modifications, especially histone-tail acetylation, have important roles in cognition, but how and why histone acetylation changes during aging and its critical role in age-related cognitive decline remain largely unknown. Here we find that the expression of two highly related epigenetic factors, the E1A-associated protein p300 (p300) and CREB-binding protein (CBP) decline in the aging mouse brain. We then used young and old $p300^{fl/fl}$ genetically modified mice in combination with adeno-associated virus-expressing Cre recombinase to generate focal homozygous deletions of p300 and CBP in the dorsal hippocampus. Our results show that the knockout of p300 and CBP causes robust impairments in the water T maze and Morris water maze tests in young mice. The impact on aging mice's learning and memory trends towards impairment but is not significant, presumably due to an already low level of p300 and CBP. Overall, these data are preliminary proof of the critical roles of histone acetyltransferase enzymes, p300 and CBP, in the molecular mechanisms underlying age-related cognitive decline and encourage the development and testing of epigenetic drugs in aging.

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Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.01

Topic: H.12. Aging and Development

Support: National Research Foundation of Korea (NRF) grant (no. 2021R1A2C2005353)
Ministry of Oceans and Fisheries, Korea (no. 201803932)

Title: Glycoproteins of Capsosiphon fulvescens modulate synaptic clustering of PSD95 and prevent social isolation-induced cognitive decline in aged male rats

Authors: *J. OH, T.-J. NAM;
Pukyong Natl. Univ., Pukyong Natl. Univ., Busan, Korea, Republic of

Abstract: Social isolation and loneliness inducing cognitive decline are serious health problems in the elderly. Although the hydrophilic glycoproteins of *Capsosiphon fulvescens* (Cf-hGP) prevent aging-induced cognitive impairment, its effects on social isolation-induced cognitive dysfunction are unclear. This study investigated the efficacy of Cf-hGP against cognitive dysfunction in aged rats and delineated its underlying mechanisms. The oral administration of Cf-hGP (15 mg/kg/d, 4 weeks) reversed the social isolation-induced decreases in phosphorylation of extracellular signal-regulated protein kinase 1/2 (ERK1/2), postsynaptic density protein 95, and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor subunit 1 and increased expression of metabotropic glutamate receptor 5 in the synaptosome of the dorsal hippocampus. Furthermore, Cf-hGP prevented social isolation-induced spatial memory impairment, and its effects were attenuated by inhibition of ERK1/2 or deglycosylation of Cf-hGP. Cf-hGP-induced clustering of ERK1/2-mediated postsynaptic density protein 95 in the dorsal hippocampus improves memory formation in socially isolated aged rats, and protein glycosylation contributes to enhancing the Cf-hGP effect.

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Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

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Program #/Poster #: 664.02

Topic: H.12. Aging and Development

Support: Center of Reconstructive Neuroscience .02.1.01/0.0./0.0/15_003/0000419

Title: The role of brain chondroitin 4 sulfates (C4S) in aging

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Abstract: Extracellular matrix (ECM), with perineuronal nets (PNNs) in particular, has played crucial role in neuronal plasticity, with impact on memory, learning and behaviour. The structures of ECM/PNN are influenced by homeostatic plasticity and changed constantly during the life time. However, two major shifts are noted; the critical period and the aging. The increase of PNNs is connected with increased incorporation of chondroitin sulphate proteoglycans (CSPGs), mainly aggrecan. This is mediated by changes in sulfation epitope of chondroitin sulfates in CSPGs. During aging, significant increase of 4-sulfation (C4S, mainly by enzyme Chst11) and decrease of "juvenile" 6-sulfation (C6S, mainly by Chst3) is being observed, associated with loss of neuronal plasticity and general cognitive decline.
In our novel mice model where Chst11 gene is conditionally knocked out in the CNS (Chst11KO) either in all neuronal lineage cells (under nestin promoter) or in parvalbumin (PV) neurons only (PV promoter), we have investigated the impact of decreased C4S/C6S ratio on learning, memory and general behaviour at 3, 6, 12 and 20 months of age. We studied the effect on long-, short-term and working memory using Morris water maze, spontaneous alternation and spontaneous novel object recognition or mice recognition tasks. Additionally, we monitored altered general behaviour by the sociability test, zero-maze and pre-pulse inhibition tests. Synaptic connectivity on parvalbumin inhibitory interneurons (Vglut/PSD95 vs Vgat/Gephyrin) and density of PNNs (WFA, aggrecan) were evaluated immunohistochemically. The hippocampal fEPSP in acute slices was measured.
We have observed significant enhancement of cognitive abilities from 3 till 20 months of Chst11KO mice. Particularly, the object novelty memory was enhanced in comparison with all control groups for short as well as long term memory. In Morris water maze, Chst11KO mice displayed increased short-term memory and reversal learning plasticity. Moreover, increased sociability and social novelty preference was observed. However, Chst11KO animals displayed increased anxiety in zero-maze and alteration in repetitive behaviour. The behavioural changes were supported by significant reduction of aggrecan incorporated into the PNNs, increased fEPSPs and alteration of synaptic connectivity on PV+ inhibitory interneurons. Our results support the restrictive role of C4S in neuronal plasticity and open possibilities of its modulation for experimental therapy of cognitive decline in late aging and neurodegenerative disorders such as Alzheimer's disease.

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Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

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Topic: H.12. Aging and Development

Support: Institute of Medical Sciences, University of Toronto
Ontario Brain Institute
Natural Sciences and Engineering Research Council

Title: Establishing an approach to recognize stress-imposed memory impairment in middle-aged mice

Authors: *A. MOUZENIAN[1], B. GEBREGERGIS[2], L. ZHANG[3], J. ROBERTSON[2];
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Abstract: Aging is associated with hippocampus-dependent memory impairment, which has been shown to be present even in middle-aged mice. Additionally, stress has been found to increase the risk of memory impairment in humans and mice. Chronic stress, particularly in middle-aged animals, should increase the rate of memory impairment. In this study, we are looking to establish an index to distinguish individual mice with impaired memory, particularly for those who have experienced chronic stress. We conducted experiments on C57BL/6J 8-11-month-old male mice. Only male mice were used to set a foundation for further assessment of sex differences in the future. We used multiple behavioural tests, including an open-field test to detect general sensorimotor function and anxiety, novel object recognition (NOR) to evaluate hippocampus-dependent memory, and fear conditioning to study memory and stress response. Open-field was assessed in a 100cm x 100cm black box for a 10-minute period. NOR included 4 days of habituation prior to the testing day and a 1-hour intertrial period between the familiarization (10 mins) and novel phase (5 mins). Over three days, fear conditioning assessed both contextual memory and cued memory by individually examining the effects of context and tone. To simulate chronic stress, mice experienced 2 hours of limited mobility in a 50 mL restraint tube for 10 days, while non-stressed mice acted as a control. We hope to establish a modified approach as per Gallagher et al., 1993 to distinguish individuals with impaired memory. We observed variability in open-field (n=22) parameters such as distance, mean speed, mobility time, and center time and variability in the results from NOR (mean recognition memory index=0.232, SD=0.269, n=19). No strong correlation (r values between -0.217 and 0.443) was found between open-field parameters and the memory index from NOR. 20 mice are currently under the stress protocol. The observed heterogeneity in performance for open-field and NOR within the control group supports other research stating memory impairment can be observed in middle-aged mice. The absence of a strong correlation indicates impairments were related to memory rather than sensorimotor impairments. We anticipate stress will further exaggerate memory impairment. The creation of this memory index should allow us to identify individual middle-aged mice with memory impairment, thus allowing for the application of positive interventions accordingly moving forward.

Disclosures: **A. Mouzenian:** A. Employment/Salary (full or part-time):; Krembil Research Institute, University Health Network, University of Toronto. **B. Gebregergis:** A. Employment/Salary (full or part-time):; Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Department of Laboratory Medicine and Pathobiology, University of Toronto. **L. Zhang:** None. **J. Robertson:** A. Employment/Salary (full or part-time):; Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Department of Laboratory Medicine and Pathobiology, University of Toronto.

Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.04

Topic: H.12. Aging and Development

Support: NIH 1RF1AG039103

Title: Recollection effects in entorhinal cortex predict longitudinal memory change in a sample of cognitively healthy older adults

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Abstract: Structural and functional variability in medial temporal lobe (MTL) regions have been reported to correlate with individual differences in episodic memory and longitudinal memory change in cognitively healthy older adults. The entorhinal cortex in particular has been reported to be sensitive to early cortical tau accumulation, and reductions in its structural and functional integrity are associated with memory decline. The current study employed manual tracing of the hippocampus (head, body and tail) and entorhinal and parahippocampal cortex to obtain volumetric measures of these regions. A sample of sixty-seven cognitively healthy older adults (aged 63- 76 years) were administered a battery of neuropsychological tests on three occasions: the second occasion one month after the first test session, and a third session three years after the first one. Memory component scores were derived from a principal components analysis of the neuropsychological test scores. To reduce retest effects, mean memory scores across sessions 1 and 2 served as a baseline for assessing change in memory performance at session 3. Fifty-five participants, all of whom had remained cognitively healthy, returned for the session 3 tests. Structural and functional MRI data were acquired between the first two sessions and included an in-scanner associative recognition procedure enabling estimation of MTL encoding and recollection effects. We previously demonstrated that encoding-related functional activity in the anterior hippocampus correlated with associative memory performance and baseline memory, while recollection-related activity correlated with associative memory performance and longitudinal memory change. Using the manually-delineated regions of interest, we identified highly robust recollection effects in the entorhinal and parahippocampal cortex. Like the effects in anterior hippocampus, entorhinal recollection effects correlated negatively with memory change, and this relationship remained after controlling for age and entorhinal cortex volume. Parahippocampal recollection effects did not correlate significantly with memory performance or memory change. The findings suggest the magnitude of recollection effects in both anterior hippocampus and entorhinal cortex in cognitively healthy older adults are sensitive predictors of longitudinal memory change.

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Poster

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Title: Red ^{630}Light Transcranial LED Therapy (RL-TCLT) reinforces the synapses in the hippocampus of aged mice and attenuates the memory loss, activating the mitochondrial function.

Authors: *C. JARA[1], D. BUENDÍA[2,3], C. TAPIA-ROJAS[1,4];
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Abstract: Over time cell damage is accumulated in the whole organism, however, the brain is particularly vulnerable to aging. In the aged brain, the hippocampus is critical for age-related memory impairment and the predisposition to the development of neurodegenerative diseases. Therefore, it is relevant to conduct studies focused on safe and non-invasive treatments to reduce the negative changes observed in the hippocampus during aging, including memory loss. Synaptic and mitochondrial dysfunction are early events during aging, both are regulated reciprocally and together contribute to age-associated memory loss. Thus, preventing synaptic damage by targeting mitochondria is an auspicious anti-aging therapy. A favorable but scarcely explored anti-aging method is the use of non-invasive transcranial LED therapy. Light-emitting diode (LED) therapy involves the interaction of photons with molecules in the cells. Red^{630}-light-Transcranial LED therapy (RL-TCLT) delivers photons in the brain, stimulating the cells with no thermal effects, which favors tissue repair and rehabilitation of neurological conditions, however, its impact on age-related memory impairment is poorly studied. Here, we treat the hippocampus of 7-month-old (mo) Senescence-accelerated mouse-prone 8 (SAMP8) mice with RL-TCLT for 125s daily, for 5 weeks. We reported that RL-TCLT improved the age-related spatial and object recognition memory impairment, all memories dependent on the hippocampus. Consistent with this idea, we detected increased presynaptic proteins synaptophysin (SYP) and Synapsin (SYN) levels, and decreased levels of the NR2B subunit of NMDAR; suggesting synaptic remodeling and increased synaptic function with less risk of neurotoxicity. Besides, we reported higher Arc protein levels, possibly related to enhanced synaptic plasticity. Moreover, we observed increased cytochrome c oxidase (COX) activity, and higher ATP production, accompanied by reduced

sensitivity to calcium overload in hippocampal mitochondria from treated SAMP8 mice. Finally, our results reveal an increase in PGC1-α, which will be due to increased mitochondrial biogenesis. Therefore, we propose that RL-TCLT has effects beneficial on hippocampal memory stimulating the mitochondrial function.

Disclosures: C. Jara: A. Employment/Salary (full or part-time):; Laboratory of Neurobiology of Aging, Centro de Biología Celular y Biomedicina (CEBICEM), Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile. **D. Buendía:** A. Employment/Salary (full or part-time):; Escuela de Ingeniería Civil Biomédica, Universidad de Valparaíso, Chile. **C. Tapia-Rojas:** A. Employment/Salary (full or part-time):; Laboratory of Neurobiology of Aging, Centro de Biología Celular y Biomedicina (CEBICEM), Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile, Centro Ciencia & Vida, Fundación Ciencia & Vida, Avda. Zañartu 1482, Nuñoa, Santiago, 7780272, Chile.

Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 664.06

Topic: H.12. Aging and Development

Title: Healthy adult-born neurons support successful cognitive aging

Authors: *N. BLIN[1], L. RUPPRECHT[1,2], L. CHICHUNG[2], F. FARRUGIA[1], V. CHARRIER[1], N. ABROUS[1];
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Abstract: The progressive decline of memory associated with aging varies from one individual to another ; some retain good memory performances while others suffer substantial loss. The hippocampus is essential for good memory processes and is particularly altered in individuals with decreased memory abilities. Adult neurogenesis in the dentate gyrus is a peculiar form of plasticity that plays an essential role in spatial learning and thus represents a good candidate for the resilience/vulnerability to cognitive aging as animals with preserved memory abilities show higher levels of neurogenesis at old age. In this project, we aim to determine the role of adult dentate granule neurons (adu-DGNs) born during young adulthood in preventing the development of memory deficits with age. We investigated possible mechanisms underlying their resilience such as the maintenance of proper functionality, connectivity and metabolism health in middle-aged and aged male rats whose cognitive statuses were assessed in the Morris watermaze. Using the immediate early gene Zif268 as a proxy for neuronal activity, we showed that adu-DGNs are more responsive to the learning task in old unimpaired rats (Resilient) compared to aged rats with reduced learning abilities (Vulnerable). To detect early signs of such a loss of responsiveness, we studied the connectivity and metabolism of adu-DGNs in middle-aged rats. We showed that the resilience of adu-DGNs does not involve the prevention of cellular

senescence (a non-apoptotic cellular arrest) or the avoidance of dendritic impairments, but rather the prevention of alterations in their post-synaptic density (indirect reflection of their glutamatergic innervation) and mitochondrial network of their proximal dendrites. All together, we showed a key role of adu-DGNs in the resilience to cognitive aging. The maintenance of memory abilities is linked to the preservation of their ability to respond to the learning task at old age. We showed a critical window for this preservation from middle-age onwards, at which resilient individuals avoid mitochondrial and post-synaptic impairments of their adu-DGNs, which might reflect a preservation of synaptic inputs.

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Poster

664. Learning and Memory in Aging

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Topic: H.12. Aging and Development

Support: The Canadian Consortium on Neurodegeneration and Aging
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Title: Longitudinal effects of an autobiographically salient music listening intervention on functional connectivity, white matter microstructure, and memory in early-stage cognitive decline: An fMRI study

Authors: *V. VUONG[1], C. E. FISCHER[2], N. CHURCHILL[2], M. LEGGIERI[2], M. TAU[2], L. R. FORNAZZARI[2], M. H. THAUT[1], T. A. SCHWEIZER[2];
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Abstract: Compared to other types of memory, people with dementia demonstrate remarkably preserved musical memory. Listening to a music playlist of autobiographically salient songs has been shown to have beneficial effects on cognitive performance. However, the mechanisms that underpin improvements in memory have not been elucidated. In this study, we used functional magnetic resonance imaging (fMRI) to investigate the effects of a 3-week personalized, autobiographically salient music listening intervention (1 hr/day, 5-7 days/week) on longitudinal changes in brain structure, function, and global memory in musicians (n=6) and non-musicians (n=8) with early-stage cognitive decline. Neural activity and global memory (using the Montreal Cognitive Assessment [MoCA]) were evaluated pre- and post-intervention. To assess changes in functional connectivity, white matter microstructure, and global memory associated with the intervention, a paired t-test was completed. The task-based scans showed a consistent decline in activation from pre- to post-intervention in the globus pallidus and right inferior frontal gyrus, suggesting greater neural efficiency. When examining participants based on musicianship, we observed that musicians showed less longitudinal change. Resting-state functional connectivity

analysis showed decreased connectivity between temporal and frontal network nodes, indicating long lasting effects of the 3-week intervention that went beyond the music listening context. Musicians showed more longitudinal change in functional connectivity, while non-musicians showed minimal effects. White matter microstructural analysis revealed longitudinal effects in structure. Specifically, measures of radial diffusivity were significantly reduced in the right superior corona radiata, with musicians demonstrating more reduction relative to non-musicians. Lastly, using a Wilcoxon Signed-Rank test, we found significant results on the memory subscore of the MoCA at post-intervention ($p=0.034$). Our results indicate that an autobiographically salient music listening intervention may induce neuroplastic changes in cognition and that musicianship may modulate these processes.

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Poster

664. Learning and Memory in Aging

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Title: Functional organization of the Medial-Frontal Parietal Network in older adults and its relationship with episodic memory

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Abstract: Normal aging entails highly variable cognitive, structural, and functional changes that could lead to pathological decline such as that caused by Alzheimer's Disease. Episodic memory (EM), a cognitive function that critically depends on hippocampal-cortical interactions, is especially vulnerable to aging. The hippocampus is considered part of the Medial-Frontal Parietal Network (M-FPN, also known as the Default Mode Network), which is a functional brain network not only associated with EM processes, but also known to show functional connectivity (fc) alterations in normal and pathological aging as shown by resting state fc (rs-fc) magnetic resonance imaging. Both the hippocampus and the M-FPN are thought to functionally

partition into segments and subnetworks, respectively, that may predict heterogeneous aging processes. Leading neuroimaging frameworks for the hippocampus propose anterior ("Head") and posterior ("Body" and "Tail") segments (Ritchey et al., 2015), while the M-FPN is proposed to be comprised of three functionally distinct subnetworks. The Schaefer parcellation (Schaefer et al., 2018), further divides these three M-FPN subnetworks (identified as A, B, and C) into a set of non-overlapping sections or parcels. The aim of this research is to understand the functional organization of the hippocampal segments with the M-FPN subnetworks in normal aging through a winner-take-all approach that assesses and compares the rs-fc partial correlations of each hippocampal segment to that of each M-FPN subnetwork. Subsequent analyses test whether these groupings are significantly associated with EM performance on two standardized neuropsychological measures of episodic memory. One sample t-tests performed on a group of older adults (N=136, mean age=66.51±5.84, 80 females) show: the hippocampal Head significantly correlates with M-FPN C, which comprises the Medial Temporal Lobe (mean fisher's z partial correlation=0.199, $t(135)=9.06$, $p<0.001$), followed by M-FPN B (mean fisher's z partial correlation=0.055, $t(135)=2.56$, $p=0.011$); the Body significantly correlates with M-FPN C (mean fisher's z partial correlation=0.173, $t(135)=8.07$, $p<0.001$) and M-FPN A (mean fisher's z partial correlation=0.060, $t(135)=2.92$, $p=0.004$); and the Tail significantly correlates with M-FPN A (mean fisher's z partial correlation=0.084, $t(135)=3.81$, $p<0.001$). These results support the functional heterogeneity of the hippocampus in its affiliation with the M-FPN at rest in aging. This project will help characterize the organization and stability of the hippocampus with M-FPN subnetworks, as well as the relevance to EM, in older adults.

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Poster

664. Learning and Memory in Aging

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Topic: H.12. Aging and Development

Support: Z01ES090089

Title: Automated home-cage assessment of discrimination and reversal learning in 5xFAD mice

Authors: *D. YOUNGSTROM, A. C. LETSINGER, B. J. BERNSTEIN, J. D. CUSHMAN, J. L. YAKEL;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory loss and cognitive deficits such as impaired cognitive flexibility. Studying cognitive flexibility deficits in mice typically involves reversal learning tasks that require diet restriction, extensive training periods that often occur during the light cycle (when mice typically sleep), and

stress-inducing animal handling, which are all known to impact AD pathology and reduce cognitive performance. Therefore, there is a need to eliminate these confounds and develop more reliable, translatable, high-throughput mouse memory tests. To address these limitations, we tested the sensitivity of an automated home-cage system, the CognitionWall, to detect cognitive deficits in a common mouse model of AD, the 5xFAD-B6SJL. The CognitionWall has 3 entrances. Mice must pass through the correct entrance 5 times to receive an automatically dispensed food pellet reward (i.e., fixed ratio 5 schedule). The mice undergo 2 nights of discrimination learning before the correct entrance is switched for the subsequent 2 nights of reversal learning to measure their cognitive flexibility. We tested female (N=52) and male (N=41) 5xFAD-B6SJL heterozygotes (HETs, N=50) and wildtypes (WTs, N=43) at 2 (N=24), 4 (N=29), 6 (N=18), and 9 (N=22) months of age with the CognitionWall. We found that HETs tended to commit more errors and required more trials to reach the 80% discrimination and reversal criteria than WTs for most age groups in male and females, which is suggestive of the expected cognitive deficits. However, 5xFAD HETs tended to earn more food pellets due to increased total entries and more cage locomotion. Lower activity levels, and therefore fewer food pellets earned, contributed to the study removal of 19% of mice after 2 days to avoid significant weight loss. Of the removed mice, 72% were WTs and 78% were 2 or 4 months old. There were no sex differences for removal. While the CognitionWall is sensitive enough to detect genotype differences in locomotion and the number of entries necessary to meet 80% discrimination and reversal learning criteria, more mice are needed to definitively detect cognitive deficits between age groups. This automated home-cage assessment of discrimination and reversal learning could be useful in preclinical studies because it is high-throughput and eliminates traditional cognitive testing confounds such as diet restriction, stress, and disrupted sleep cycles.

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Poster

664. Learning and Memory in Aging

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Topic: H.12. Aging and Development

Support: ARC-NL
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Title: Impairment of olfactory fear extinction in aged rats

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Abstract: Aging is associated with a decline in cognitive function, affecting associative learning and memory formation. Previously, we have shown a shift from NMDAR- to L-type calcium

channel (LTCC)-dependent long term depression (LTD) in the aged piriform cortex. However, the functional relevance of LTCC-LTD in aged animals is unknown. LTD has been associated with behavioral flexibility such as learning extinction. Here we compare the extinction of a learned olfactory-associated behaviour in young and aged rats. Two groups of mixed-gender SD rats, young (2 months old) and aged (22 months old) underwent olfactory fear conditioning using an odor (conditioned stimulus), and a foot shock, (unconditioned stimulus). Following successful learning, rats freeze significantly more in response to the conditioned odor compared to the no-odor baseline. Following fear learning, rats underwent 7 days of extinction. During each day of extinction, freezing time in response to the odor was measured and compared between young and old groups. Using ex vivo electrophysiology in slices containing the PC, we compared LTD in the associational fiber layer in young and aged rats following the 7th day of extinction. In young rats, over the course of the 7-day extinction period, we observed successful fear extinction measured by a reduction in freezing time. However, aged rats showed little behavioral flexibility and impaired extinction of the freezing behaviour. In parallel, we observed a consistent PC LTD in aged rats following the extinction protocol, whereas no LTD was observed in young rats. Our data suggests that aged rats may have a deficit in extinction of fear driven, odor-associated memories. It has been reported that olfactory associative learning increases the preposition of LTD in rat PC. Here we show extinction is accompanied by decreased LTD in young rats, whereas impaired extinction in aged rats is associated with inducible LTD. PC LTD may be a neural correlate for olfactory extinction learning.

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Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.11

Topic: H.12. Aging and Development

Support: 1R56AG060052-01

Title: Age-related dedifferentiation in occipital and temporal regions during episodic memory task performance

Authors: *P. SKOLASINSKA, E. T. SMITH, S. QIN, G. H. SHARARD, F. SIERRA, E. MANON, E. NGUYEN, C. BASAK;
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Abstract: Healthy aging is related to decreased ability in discrimination between items previously seen and those that are similar (i.e., pattern separation). In this fMRI study, episodic memory task (MST) performance and fMRI-BOLD signal during encoding, were compared between a group of 43 older (M_{age} = 71.5, SD_{age} = 4.8) and 24 younger (M_{age} = 24.7, SD_{age} = 3.8) adults. During in-scanner encoding, participants engaged in incidental learning of objects.

During out-of-scanner recognition, participants indicated if objects were “old”, “similar”, or “new”. Both the Recognition (REC) and the Lure Discrimination Index (LDI) measures were obtained. In line with previous research, while the groups did not differ in REC, older adults had significantly lower LDI, than young. Whole-brain fMRI analysis was used to identify age-sensitive regions of activation. A single task-positive (Remember>Forgot) cluster was found which included the left lateral occipital and temporal regions, parahippocampal gyrus and the hippocampus. Younger adults engaged this region exclusively during the remembered trials, showing a positive correlation between BOLD signal and LDI. In contrast, older adults engaged this region indiscriminately during all conditions, with no relation to performance. Current study provides evidence for age-related dedifferentiation in brain regions crucial for successful pattern separation.

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Poster

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Title: Synaptic resilience in Lothian Birth Cohort 1936 participants is associated with maintained cognition during ageing

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Abstract: Many people experience declining cognitive function as the brain ages. Age is the most important risk factor for cognitive decline and dementia, but the degree to which individuals experience these aspects of ageing is hugely variable. Understanding the neurobiological bases of brain ageing can offer key insights into individual differences in cognitive ageing and dementia risk. Region-specific synapse changes observed in the ageing brain and in Alzheimer's disease (AD) correlate with cognitive decline; thus, we hypothesize that synaptic resilience contributes to healthy cognitive ageing. In this study, we test this hypothesis through observing human post-mortem brain tissue from people without dementia donated by participants in the Lothian Birth Cohort 1936 alongside young control subjects and people who died with AD. We used array tomography imaging to examine synapse density and the accumulation of Alzheimer's-related pathological proteins within synapses in two brain regions (inferior temporal cortex and visual cortex). Further, we use proteomics and RNA sequencing to characterise molecular changes in biochemically enriched synaptic fractions and in total brain homogenates from these brain regions. We observe a stepwise decline in synapse density between young controls, ageing participants with normal cognition, and people with AD and a stepwise increase in the proportion of remaining synapses containing amyloid beta or tau proteins. Molecular analyses indicate decreases in synaptic signalling in ageing compared to young controls, which are exacerbated in AD. The Lothian Birth Cohort 1936 participants took an intelligence test at age 11 and multiple cognitive tests through their 70s and 80s allowing us to compare people with lifetime cognitive decline compared to those with maintained or resilient cognition over their lifetimes. Although our participant numbers are modest when split by lifetime cognition (n= 7 participants who experienced lifetime cognitive decline and 8 with lifetime cognitive resilience), we observe some differences, including increased gliosis in people with lifetime cognitive decline and paradoxically gene changes indicating decreased synaptic signalling in people who were cognitively resilient despite no change in synapse density. iPSC neurons from LBC1936 participants with lifetime cognitive resilience also exhibit dampening of synaptic genes in response to challenge with aged human brain homogenate containing Aβ compared to neurons from people with lifetime cognitive decline. Together, our data indicate that synaptic resilience may play an important role in maintaining cognition during ageing.

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Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 664.13

Topic: H.12. Aging and Development

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Title: Unsupervised, frequent and remote: a novel platform for personalised digital phenotyping of spatial working memory and image recognition in humans.

Authors: M. BAUZA[1], ***M. KRSTULOVIC**[2], J. KRUPIC[2];
[1]Univ. Col. London, London, United Kingdom; [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: AIMS: Spatial working memory and image recognition tests are commonly used to facilitate the diagnosis of hippocampal-related neurological disorders such as Alzheimer's disease. With the growing ageing population healthcare systems are struggling to meet the increasing demand of diagnostic testing for such neurological disorders. Hence, there is an urgent need to implement remote, ideally completely unsupervised neurological testing to facilitate early diagnostics and improve patient stratification.
METHODS: We developed a novel digital game-like platform, hAge ('healthy Age'), which incorporates spatial alternation, image recognition and a visuospatial task for the frequent, remote and unsupervised assessment of spatial and non-spatial working memory. Overall, 191 healthy adults were tested who continuously engaged with the hAge ~10 times/day for eight weeks or longer. Participants were split into three groups: young (18-44), middle-aged (45-64), and older (65>).
RESULTS: We report a 65% adherence level, similar to other unsupervised platforms. Consistent with findings from analogous standard laboratory-based tests our data shows, that generally participants found the visuospatial task to be more challenging than the image recognition task and that performance on the spatial alternation task negatively correlated with inter-trial periods. Unexpectedly, we found that the oldest group outperformed the young and the middle-aged group in all tasks. Importantly, we observed that the performance of the middle-aged and older group of participants improved significantly with experience. Secondly, we observed that the older group of participants had the most difficulty adjusting to the different choice types in the double spatial alteration task, in comparison to the younger and middle-aged groups.
CONCLUSIONS: The observed strong practice effect in the middle-aged and older participant groups establish the validity of our approach as they have been previously identified as measures

of cognitive decline in patients with Mild Cognitive Impairment in similar spatial tasks. Furthermore, our results show that older participants exhibit a significant difference in performance in different types of choices in the double spatial alteration task. Older participants performing better than young participants, while unexpected, can be explained by unrelated confounds affecting performance which highlight challenges related conducting large-scale uncontrolled testing.

Disclosures: **M. Bauza:** None. **M. Krstulovic:** None. **J. Krupic:** None.

Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.14

Topic: H.12. Aging and Development

Title: A pilot study investigating a voice recognition cognitive screening tool for detection of neuropsychological change

Authors: K. PATIL, J. ROSEN, ***L. H. ALASANTRO**, A. KAUP, G. WAGNER, V. VIGNISSON, G. SAHAGIAN;
The Neurol. Ctr. of Southern California, Carlsbad, CA

Abstract: Background: The prevalence of mild, age-advanced changes in cognition, defined as mild cognitive impairment, is spiking as the baby boomer population ages. Early and accurate detection of age-advanced changes in thinking abilities leads to proper care, efficient use of resources, and promotion of research. Indeed, government-based health care systems and insurance programs incentivize routine neurocognitive assessment. Brief, paper and pencil cognitive screeners such as the MMSE and MOCA are widely utilized in clinical settings. Such screeners lack sensitivity to subtle cognitive change and specificity related to the cause of decline. Moreover, administration and interpretation of these screeners is time consuming in a busy, clinical setting. Prior research has determined that electronic-based testing is effective in detecting cognitive change (Zygouris & Tsolaki, 2014). This pilot study aimed to investigate a convenient, platform agnostic, voice-recognition screening tool for detection of mild, age-advanced neurocognitive problems. **Method:** Participants (n=25) were recruited from a large community-based neurology practice, who underwent a comprehensive neuropsychological (NP) evaluation as part of their standard of care. Participants were administered an auditory list learning/recall test and a verbal fluency test (F/A/S and animals) via a voice recognition cognitive screening tool. Pearson correlations were conducted to examine the concurrent validity between the standard NP measures and those obtained using the current screening tool. **Result:** Preliminary data suggests a strong, positive correlation between standard NP measures and those obtained using voice recording and speech recognition technologies; language: F/A/S ($r = 0.65$, $p < 0.05$), animals ($r = 0.67$, $p < 0.05$); delayed word list recall Z-scores ($r = 0.53$, $p < 0.05$). **Conclusion:** This pilot study demonstrated concurrent validity between standard NP measures of

delayed word list recall and verbal fluency and those obtained using a novel, voice recording and speech recognition screening tool. These initial data provide support for the value of a computerized based screening tool for older adults. Such a screening too has the potential to maximize utilization across multiple settings (e.g., medical office, hospital, in-home) and improve early detection of cognitive decline.

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Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.15

Topic: H.12. Aging and Development

Support: NU Office of Undergraduate Research

Title: Enriched environment reversed learning and memory deficit of genetically stress reactive female WMI rats at middle age

Authors: *M. NEMESH, M. T. JI, K. J. PRZYBYL, A. M. HARTER, C. S. KIM, A. YAMAZAKI, E. E. REDEI;
Psychiatry and Behavioral Sci., Northwestern Univ., Chicago, IL

Abstract: Declining memory in middle age, either as a precursor or a predictor of later age dementia, remains a quality-of-life impairment. Accumulation of aging-related pathology in the brain can reach a certain threshold, usually during middle age, triggering different biological changes that may lead to cognitive decline. In the absence of any disease-modifying treatments, there is increasing focus on primary prevention to reduce the risk of cognitive decline, and on early intervention to slow progression of this process. Environmental enrichment is shown to enhance learning and memory and attenuate age-induced learning and memory deficit. This study aimed to investigate if environmental enrichment (EE) would attenuate age-induced learning and memory deficit in a genetic rat model of heightened stress reactivity that also shows memory deficit at middle age. The near-isogenic Wistar Kyoto Less Immobile (WLI) and Wistar Kyoto More Immobile (WMI) male and female rats were tested for their hippocampus-dependent learning and memory at 6 months (6M), and 12 months (12M) of age. The third group consisted of male and female WLI and WMI rats that were placed into EE for six months, from 6 to 12 months of age, and tested then for their cognitive functions. Learning and memory were assessed by Contextual Fear Conditioning (CFC) and Morris Water Maze (MWM) tests for all groups. Contextual fear memory declined significantly in both male and female 12M WMIs, but not in WLIs. The 12M EE females regained their memory completely, showing fear memory comparable to that of 6M old animals. In contrast, EE had no effect on the 12M male WMIs or male and female WLIs in their freezing response at the second day of CFC, which serves as the

measure of fear memory. The latency to reach the platform in the MWM showed a very similar pattern. By the last trial of the fourth day, 12M EE WMI females had no learning deficit as opposed to 12M WMI females. The 12M EE WMI males tended to increase their performance as well. Plasma corticosterone levels were elevated in the 12 EE animals in general, particularly in the females. Since increased plasma corticosterone levels were found in the WLI females and males as well, the EE-induced reversal of learning and memory deficit in middle-aged WMI females is not likely to be related to their corticosterone levels. Considering that EE reversed the memory deficit in the more stress-reactive WMI females, the hypothesis that EE functions via inoculating against subsequent stressors (such as the tests) could explain why memory deficit was reversed only in this group by environmental enrichment.

Disclosures: M. Nemesh: None. **M.T. Ji:** None. **K.J. Przybyl:** None. **A.M. Harter:** None. **C.S. Kim:** None. **A. Yamazaki:** None. **E.E. Redei:** None.

Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.16

Topic: H.12. Aging and Development

Support: JP19K16985
LEOC Co

Title: Mindfulness intervention improves cognitive function in older adults by enhancing the level of miRNA-29c in neuron-derived extracellular vesicles

Authors: *M. NAKANO, S. HASHIZUME, K. KUBOTA, E. KOBAYASHI, M. FUJIMIYA; Sapporo Med. Univ. Dept. of Anat., Sapporo Med. Univ. Dept. of Anat., Sapporo City, Hokkaido, Japan

Abstract: Although mindfulness-based stress reduction (MBSR) improves cognitive function, the mechanism is not clear. In this study, people aged 65 years and older were recruited from elderly communities in Chitose City, Japan, and assigned to a non-MBSR group or a MBSR group. Before and after the intervention, the Japanese version of the Montreal Cognitive Assessment (MoCA-J) was administered, and blood samples were collected. Then, neuron-derived extracellular vesicles (NDEVs) were isolated from blood samples, and microRNAs, as well as the target mRNAs, were evaluated in NDEVs. A linear mixed model analysis showed significant effects of the MBSR x time interaction on the MoCA-J scores, the expression of miRNA(miR)-29c, DNA methyltransferase 3 alpha (DNMT3A), and DNMT3B in NDEVs. These results indicate that MBSR can improve cognitive function by increasing the expression of miR-29c and decreasing the expression of DNMT3A, as well as DNMT3B, in neurons. It was also found that intracerebroventricular injection of miR-29c mimic into 5xFAD mice prevented cognitive decline, as well as neuronal loss in the subiculum area, by down-regulating Dnmt3a

and Dnmt3b in the hippocampus. The present study suggests that MBSR can prevent neuronal loss and cognitive impairment by increasing the neuronal expression of miR-29c.

Disclosures: **M. Nakano:** None. **S. Hashizume:** None. **K. Kubota:** None. **E. Kobayashi:** None. **M. Fujimiya:** None.

Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.17

Topic: H.12. Aging and Development

Support: NIH Grant P01AG009973
NIH Grant UH2/3NS101856
NIH Grant R44AG063607

Title: BPN-27473: a novel $GABA_A\alpha5$ positive allosteric modulator that improves memory performance of rats with conditions of hippocampal hyperactivity

Authors: *M. KOH[1], D. L. DAVIES[2], L. ASTRAYAN[2], J. LIANG[2], Q. JIANG[3], M. HACHICHA[4], S. ROSENZWEIG-LIPSON[5];
[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Sch. of Pharm., USC, Los Angeles, CA; [3]Curia Global, Albany, NY; [4]Div. of Translational Research, Blueprint Neurotherapeutics Program, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [5]AgeneBio, Baltimore, MD

Abstract: Excitation-inhibition imbalance in the hippocampus is a major contributor to cognitive impairment in aging and in schizophrenia. The imbalance, characterized by weakened inhibition, is thought to induce an aberrant condition of increased neural activity observed in the hippocampus. Inhibitory $GABA_A$ α5 receptors are localized in the hippocampus and play a critical role in regulating hippocampal activity. We investigated whether boosting hippocampal inhibition via activation of $GABA_A$ α5 receptors could normalize neural overexcitability in the hippocampus and improve hippocampal-dependent cognition in animal models of aging and of schizophrenia. BPN-27473 is a highly potent, selective, and orally active $GABA_A$ α5 positive allosteric modulator (PAM). It demonstrates high binding and functional selectivity at $GABA_A$ α5 over $GABA_A$ α1, with no evidence of PAM activity at α1 up to 1 μM. BPN-27473 demonstrates *in vivo* $GABA_A$ α5 receptor occupancy in the hippocampus of both male and female rats. Using radial arm maze and water maze tasks, we found that BPN-27473 improved hippocampal-dependent memory in aged rats with memory deficit after both intraperitoneal and oral administration as well as after both acute and chronic administration. It also did not produce motor impairment at doses at or above memory function. Similarly, the compound significantly improved memory performance of young adult rats exposed sub-chronically to ketamine during adolescence that models the cognitive and pathophysiological phenotypes of schizophrenia.

Additionally in a small pilot study, BPN-27473 was effective in reducing hippocampal neural activation induced by systemic administration of a small dose of kainic acid in normal rats to mimic hippocampal overactivity. Taken together, our data provides strong evidence supporting the use of $GABA_A$ α5 PAMs to reduce hippocampal overactivity and to treat mild cognitive impairment due to Alzheimer's disease and cognitive impairment associated with schizophrenia.

Disclosures: M. Koh: None. **D.L. Davies:** None. **L. Astrayan:** None. **J. Liang:** None. **Q. Jiang:** A. Employment/Salary (full or part-time):; Curia Global. **M. Hachicha:** None. **S. Rosenzweig-Lipson:** A. Employment/Salary (full or part-time):; AgeneBio. 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.; AgeneBio.

Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 664.18

Topic: H.12. Aging and Development

Title: Older Age Moderates Associations Between White Matter Network Properties and Cognition

Authors: *M. MEIRING[1], S. DÜZEL[3], S. KÜHN[4], A. BENDER[2];
[1]Neurosci., [2]Epidemiology, Michigan State Univ., East Lansing, MI; [3]Lifespan Psychology, [4]Envrn. Neurosci., Max Planck Inst. for Human Develop., Berlin, Germany

Abstract: Older age is associated with microstructural changes in the white matter (WM) connections that constitute the brain's structural network. Advanced age is also associated with altered WM network properties, including increased network segregation, or local specialization and with reduced network integration reflecting connectivity between more disparate parts of the network. Limited findings have related age-associated differences in WM network parameters (NPs) with concurrent cognitive deficits. However, whether older age attenuates or moderates these associations has not been previously evaluated. We hypothesized different patterns of associations between WM NPs and performance on age-sensitive cognitive tasks for young and older adults. Study participants (n=392; 39% female) included younger (n=99; mean age=30.85, sd= 3.56 years) and older adults (n=293; mean age=70.34, sd=3.92 years) drawn from the Berlin Aging Study-II with cognitive test data and who also underwent MRI scanning. Connectomic analysis used cortical and subcortical regions parcellated from structural MRI with the anatomically constrained tractography method in MRtrix to estimate adjacency matrices from native space tractography. NPs included measures of integration like path length (lambda), mean degree, and global efficiency, and segregation such as clustering coefficient, Q modularity, and small-world. We fit separate linear regressions of cognitive measures (global reading speed, recognition memory, 2-back, and composites of Speed and GF) on individual NPs, and

covariates Age group (AG), Sex, and AG × NP, followed by separate post-hoc regressions by AG. Overall, higher Q modularity predicted lower Speed, GF, and reading speed. Higher efficiency predicted lower reading speed, while higher community structure predicted lower recognition. Speed revealed a significant negative interaction of AG × degree. Whereas higher mean degree predicted faster speeded processing for young adults, we found the opposite for older adults. Verbal recognition had a significant negative interaction of AG × efficiency, and a positive interaction for AG × clustering coefficient. Whereas higher efficiency and lower clustering coefficient predicted better recognition in young, the opposite pattern was significantly manifest in older adults. Thus, these results show WM network integration and segregation are not equivalently associated with cognitive performance in younger and older adults. Although further work is needed, these findings highlight the role of WM network remodeling as a potential correlate or mechanism of age-associated cognitive resilience.

Disclosures: **M. Meiring:** None. **S. Düzel:** None. **S. Kühn:** None. **A. Bender:** None.

Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.19

Topic: H.12. Aging and Development

Support: CIHR

Title: The effect of chronological age and menopause on resting state connectivity and episodic memory in women.

Authors: *S. LOPARCO[1], S. RAJAGOPAL[2], S. PASVANIS[2], B. MISIC[3], N. RAJAH[2]; [1]McGill Univ. Integrated Program in Neurosci., Montreal, QC, Canada; [3]Montreal Neurolog. Inst., [2]McGill Univ., Montreal, QC, Canada

Abstract: There is growing evidence that age-related decreases in episodic memory arise at midlife, a time when females menopause. For some, menopause is associated with memory changes, raising the question of how reproductive aging interacts with chronological age effects on memory and brain function. Though a few studies have investigated menopausal effects on brain function during specific task performance, no study to our knowledge has investigated menopausal effects on the intrinsic functional organization of the brain at rest. In the current study we explored how age and menopause status related to whole-brain patterns of resting-state functional connectivity in a sample of 82 females, 21 to 65 years of age (Mean age= 42, N=57 pre-menopausal, N = 25 post-menopausal) using multivariate behavioral partial least squares (PLS) connectivity analysis. We examined if the relationship between chronological age and whole-brain functional connectivity differed between pre- and post-menopausal participants, and if these connectivity patterns related to performance on a face-location source memory paradigm. Robust regressions predicting source memory retrieval from age and menopausal status revealed

that menopause was a stronger predictor of memory performance, compared to age. The PLS connectivity results indicated that only in post-menopausal women, connectivity of visual network with hippocampus, dorsal and ventral attentional networks, and the somato-motor network increased with age and was negatively correlated with source memory performance. In contrast, greater between network connectivity amongst visual, control, attentional and somato-moter networks was positively correlated with better source memory performance in middle-aged and young pre-menopausal women, and negatively correlated with age in pre-menopausal middle-aged women. Therefore, menopause is associated with changes in source memory performance in females, and this relates to different patterns of resting state connectivity amongst visual, hippocampal and higher order attentional control networks for post- compared to pre-menopausal women at midlife.

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Poster

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Topic: H.12. Aging and Development

Support: National Institute of General Medical Sciences / National Institutes of Health,grant R01GM128183

Title: Propofol attenuates surgery-induced neuroinflammation, apoptosis, and cognitive impairment in aged mice

Authors: *R. NAGARAJAN[1], J. LYU[2], M. KAMBALI[1], M. WANG[2], R. A. PEARCE[3], U. RUDOLPH[1];
[1]Dept. of Comparative Biosci., Col. of Vet. Medicine, Univ. of Illinois at Urbana-Champaign,, Urbana, IL; [2]Dept. of Comparative Biosci. and Neurosci. Program, Col. of Vet. Med. , Univ. of Illinois at Urbana-Champaign, Urbana, IL; [3]Dept. of Anesthesiol., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Postoperative cognitive dysfunction (POCD) following surgery and anesthesia is unfortunately still quite common. It increases morbidity and mortality, especially in aging populations. Recent studies suggest that apoptosis and activation of microglia and neuro-glial interactions may be key mechanisms in POCD. Propofol, the most commonly used intravenous anesthetic, acts primarily via $GABA_A$ receptors. However, it has also been suggested as a useful anti-inflammatory adjunct for patients undergoing coronary artery bypass graft (CABG) surgery. Furthermore, it has been shown to have neuroprotective effects in aged mice and Alzheimer's disease mouse models. Here, we set out to assess the impact of chronic intermittent propofol on postoperative cognitive deficits in aged mice. Abdominal surgery under isoflurane anesthesia

was performed in 21-24 months-old mice. Animals received either chronic intermittent propofol (CIP, 75 mg/kg i.p.) or vehicle (Intralipid^{R}) every 5th day throughout the experiment. CIP led to a sustained redistribution of the $GABA_A$ receptor α5 subunit to the surface membranes, as reported previously by others for etomidate (AA Zurek et al., J Clin Invest 2014;124:5437-5441). Laparotomy performed on day 17 impaired learning and memory functions, as determined using a behavioral test battery that included Y maze alternation, novel object recognition, water maze learning and reversal learning and cued and contextual fear conditioning, compared to no surgery controls. Strikingly, CIP strongly attenuated the surgery-induced memory impairment. Western blots on hippocampal tissues showed that abdominal surgery promoted apoptosis, as evidenced by increased expression of cleaved caspase-3, cleaved caspase-9, and Bax. Moreover, surgery increased expression of Iba-1 (a marker of microglial activation) in aged mice, but chronic intermittent propofol prevented this apoptotic and microglial activation. Our results suggest that propofol - via mechanisms not fully understood - improves cognitive function by attenuating surgery-induced neuroinflammation and caspase activation. These findings support a therapeutic potential of perioperative propofol in reducing the risk of surgery-induced cognitive decline in aged individuals.

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Poster

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Topic: H.12. Aging and Development

Support: Brown Foundation

Title: Varying doses of Ibuprofen induced differing effects on memory, cognitive flexibility, and GluN1 and C-terminal splice variants expression across sex and age

Authors: *I. ABOU-SEADA, M. FRISCHMAN, K. KIM, D. KULKARNI, E. SACKINGER, K. MAGNUSSON;
Oregon State Univ., Corvallis, OR

Abstract: Ibuprofen is a commonly used non-steroidal anti-inflammatory drug. Drugs like this reduce risk of Alzheimer's disease development, but interventional studies have neutral or negative impacts on disease progression. In the current study we tested the effects of varying ibuprofen doses on cognitive functions across aging in male mice and across sexes in young mice. To do this both male and female young mice (2 month; N=6) and male older mice (23-24 month; N=6-7) were administered NIH-31M chow with either 0, 375, or 1000ppm of ibuprofen. Memory and cognitive flexibility were tested with the use of the Morris water maze, and results were analyzed based on dosage and separately for age or sex. Results indicated that at high

dosages (1000ppm or 5.8 pills/day for humans) ibuprofen impaired long-term memory in older males. Ibuprofen was found to improve cognitive flexibility and other functions in young males, but had little effect on females. Western blotting was then used to try to relate these behavioral results with the altered expression of all GluN1 subunits or C-terminal splice variants of the N-methyl-D-aspartate (NMDA) receptor in synaptic and extrasynaptic membranes of the frontal cortex and hippocampus. No significant differences in all GluN1 subunits or splice variants expression were seen in frontal cortex synaptic membranes. Frontal cortex extrasynaptic membranes: Young females expressed all GluN1 subunits at higher levels than young males when fed 0 ppm ($p=0.04$). For young males, 1000ppm ibuprofen increased GluN1 subunit expression compared to 0 ppm ($p=0.05$). In older males, we observed that 375 ppm of ibuprofen increased C1 ($p=0.02$) and C2 ($p=0.04$) splice cassette expressions compared to those fed 0 ppm. Hippocampal synaptic membranes: Young males fed 375 ppm ibuprofen had significantly increased C2 splice cassette expression when compared to both 0 ($p=0.03$) and 1000 ppm ($p=0.02$). This bell-shaped curve pattern was also evident in the GluN1 subunit expression for young males. Interestingly this bell-shaped pattern also appeared for the C1 and C2 splice cassettes in frontal cortex extrasynaptic membranes, but for older males not younger ones. The behavioral results indicated that ibuprofen use, especially in older males, can be detrimental to long term memory. These results further suggested that ibuprofen may impact cognition not only by affecting GluN1 subunit expression, but also by altering splicing and trafficking. It is important to determine the extent to which ibuprofen causes these NMDA receptor changes, specifically in older males, due to ibuprofen's use by so many of the elderly population.

Disclosures: I. Abou-Seada: None. **M. Frischman:** None. **K. Kim:** None. **D. Kulkarni:** None. **E. Sackinger:** None. **K. Magnusson:** None.

Poster

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Program #/Poster #: 664.22

Topic: H.12. Aging and Development

Support: Brown Foundation

Title: Chronic administration of ibuprofen in mice induced differential changes in memory, cognitive flexibility, and GluN2A and GluN2B protein expression across sex and age

Authors: *M. FRISCHMAN, I. ABOU-SEADA, K. KIM, D. KULKARNI, E. P. SACKINGER, K. R. MAGNUSSON;
Oregon State Univ., Corvallis, OR

Abstract: Young (4 mo) male and female and older (26 mo) male C57BL/6 mice were chronically administered 0, 375, or 1000ppm ibuprofen in NIH-31M chow and tested for memory and cognitive flexibility with the use of a Morris water maze. A dose-related response

indicated that worsening memory with ibuprofen administration was a sex-linked response, wherein males showed poorer performance compared to female mice. Young male mice on ibuprofen, however, showed improved flexibility and executive function. With the use of Western blotting, we explored whether these behavioral differences were associated with altered protein expression of N-methyl-D-aspartate (NMDA) receptor subunits, GluN2A and GluN2B, in the synaptic or extrasynaptic membranes from hippocampus and frontal cortex. In older male mice, 1000ppm ibuprofen increased synaptic GluN2A expression in the frontal cortex compared to young males on the same dose (p=.026). In young female mice, 375ppm ibuprofen decreased extrasynaptic GluN2B expression in the frontal cortex, compared to 0 ppm (p=.038). In the hippocampus, 375ppm ibuprofen in young male mice showed an increase in synaptic GluN2A expression, compared to chow fed mice. These results suggest that ibuprofen may not only have affected NMDA receptor protein expression, but also trafficking. The behavioral results showed cause for concern in chronic ibuprofen usage, which is common amongst roughly one-third of the US elderly population. Similarly, changes in NMDAR subunit composition could contribute to disease pathology and cognitive impairment. This research creates an imperative for further research to determine the extent to which ibuprofen poses a risk for exacerbated memory declines, particularly in elderly males, given its large popularity in the US.

Disclosures: **M. Frischman:** None. **I. Abou-Seada:** None. **K. Kim:** None. **D. Kulkarni:** None. **E.P. Sackinger:** None. **K.R. Magnusson:** None.

Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 664.23

Topic: H.12. Aging and Development

Support: NIH RF1AG028271
NIH R03AG067061

Title: Short-term high-fat diet consumption impairs long-term potentiation in the aged hippocampus

Authors: *B. M. GONZALEZ OLMO[1,2], M. BETTES[2], J. DEMARSH[2], F. ZHAO[3], C. ASKWITH[3], R. M. BARRIENTOS[2,4,5];
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Abstract: More Americans are consuming diets higher in saturated fats and refined sugars than ever before. These trends could have serious consequences for the older population because high-fat diet (HFD) consumption, known to induce neuroinflammation, has been shown to aggravate and accelerate memory declines. Because obesity is a complex disease with many

comorbidities, making the study of underlying mechanisms difficult and confounded, we employ a short-term diet manipulation protocol. We have previously demonstrated that short-term consumption (3 days) of a HFD among aged rats produced profound impairments to contextual and emotional/fear memories, which depend on an intact hippocampus and amygdala. These impairments were precipitated by increases in proinflammatory cytokines, primarily interleukin-1 beta (IL-1B), in both brain regions. Here, we explore the extent to which HFD consumption amongst aged rats disrupts hippocampal long-term potentiation (LTP), the form of synaptic plasticity thought to underlie long-term memory consolidation in mammals. Furthermore, we explore whether these effects could be reversed with an IL-1 receptor antagonist (IL-1ra). Young adult (3 months old) and aged (24 months old) F344xBNF1 rats were assigned to either chow or HFD for 3 days. Using transverse hippocampal slices, we examined the individual and combined effects of age and diet on several forms of synaptic activity. Specifically, excitatory post-synaptic potentials were induced in the stratum radiatum of CA1 and LTP expression was triggered with a theta-burst stimulation protocol. Our preliminary data demonstrate that late-phase LTP was particularly compromised by the combination of aging and HFD while LTP maintenance was robust in chow-fed young and aged rats. Results of IL-1ra treatment on these effects will be presented. These findings suggest that the previously observed neuroinflammation-mediated hippocampal memory impairments in aged HFD-fed rats occurs, at least in part, through deterioration of synaptic plasticity, as measured by LTP, in the hippocampus.

Disclosures: B.M. Gonzalez Olmo: None. **M. Bettes:** None. **J. DeMarsh:** None. **F. Zhao:** None. **C. Askwith:** None. **R.M. Barrientos:** None.

Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 664.24

Topic: H.12. Aging and Development

Support: NIH Grant T32HL007446
NIH Grant R25NS115552
NIH Grant P30-AG-013319-27 sub award

Title: Water T-maze spatial learning test to assess cognitive flexibility in aged mice

Authors: S. M. GREENE[1,2], G. A. ALCALA[1,2], I. BUSTAMANTE[1,3], B. BORDAS[1,4], A. JOHNSON[1,5], *G. G. GOULD[1];
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Abstract: Rodent cognitive flexibility is often assessed through spatial learning in the Morris water maze. However, for mice fatigue may introduce a potential confound in these tests, so innovative researchers introduced the water T-maze for mice as a less exhausting alternative spatial learning task (Guariglia SR and Chadman KK. J Neurosci Methods. 2013;220:24-9). Fatigue in swim tasks is an important consideration for aged mice, and in prior studies cognitive flexibility deficits in the Morris water maze emerged in aged C57BL6 mice. We compared performance of male and female 2-month, 9-15-month, and 24-month-old mice in the water T-maze test, hypothesizing that similar deficits to those seen in the Morris water maze would emerge. For spatial acquisition we filled water T-mazes with 2.5 L water made opaque by 10 ml white tempra paint. To determine initial preference, each mouse was placed in the maze and the first arm it swam to was noted. A sunken platform was placed in the opposite arm of the initial preference, and the time to find the platform and number of incorrect arm entries were noted. This step was repeated for all mice, with 10 rounds of platform acquisition per day for 5 consecutive days. To measure cognitive flexibility, the sunken platform was moved to the opposite training arm, and the process was repeated. On reversal learning day 1, the most important day for assessing cognitive flexibility, impairments in performance were only observed in one 25-month-old female mouse that appeared to have cataracts. Mice were tested for two more days after reversal, and age-based differences did not emerge. This outcome shows the water T-maze is similarly performed by young versus aged mice. This test could prove useful for testing cognitive flexibility deficits in mouse models of diseases associated with aging, such as Alzheimer's and Parkinson's disease, to reduce potential confounds from fatigue or motor deficits.

Disclosures: S.M. Greene: None. **G.A. Alcala:** None. **I. Bustamante:** None. **B. Bordas:** None. **A. Johnson:** None. **G.G. Gould:** None.

Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 664.25

Topic: H.12. Aging and Development

Support: NIH Grant RF1AG028271
NIH Grant R03AG067061
NIH Grant R21AG071135
NIH Grant T32NS105864

Title: Short-term consumption of a high-fat diet makes post-operative cognitive dysfunction persistent & induces anxiety-like behaviors in aged male rats

Authors: *S. MUSCAT[1], N. DEEMS[1], M. BUTLER[3], R. M. BARRIENTOS[2];
[1]Ohio State Univ., [2]The Ohio State Univ., The Ohio State Univ., Columbus, OH; [3]Ohio State Univ., Ohio State Univ., Columbus, OH

Abstract: Background: Gradual declines in cognition are associated with normal aging but can turn precipitous following peripheral immune insults. Post-operative cognitive dysfunction (POCD) is an abrupt decline in neurocognitive function—including difficulties with executive functions, memory impairment, confusion- experienced by some older individuals following surgery, that persists days to months after surgery. Importantly, longer-lasting POCD can develop into dementia. Advanced age is the biggest risk factor, but specific mechanisms remain unknown. To date, preclinical models largely fail to recapitulate persistent POCD. Clinically, obesity & other comorbidities linked to high-fat diet (HFD) consumption are identified as risk factors for POCD, although underlying mechanisms remain unclear. We've previously shown that short-term (3d) HFD consumption evokes neuroinflammation and induces hippocampus- & amygdala-dependent memory deficits in aged rats. Therefore, we hypothesized that HFD consumption before surgery would induce exaggerated neuroinflammation & persistent memory deficits in aged rats. **Methods:** Young (3mo) & aged (24mo) rats were fed chow or HFD for 3d immediately before receiving sham surgery or laparotomy (exploratory abdominal surgery). 2wks later, rats underwent contextual fear conditioning to assess hippocampus- & amygdala-dependent long-term memory and open field to assess anxiety-like behavior. **Results:** Aged rats who received both HFD & laparotomy had impaired hippocampus-dependent memory compared to all other groups. Aged animals fed HFD exhibited impaired amygdala-dependent memory independent of surgery condition. Interestingly, young adult rats fed HFD before surgery experienced amygdala-dependent memory impairment. Open Field behavior revealed that HFD alone induces anxiety-like behavior in aged males. Assessment of neuroinflammatory markers is ongoing. **Conclusions:** These behavioral findings suggest that HFD may 1) increase risk of persistent POCD-associated memory impairments following surgery in aged rats, 2) make young adult rats vulnerable to amygdala-dependent memory dysfunction following subsequent immune challenge, and 3) these memory deficits are independent of HFD-induced anxiety-like behaviors independent of secondary immune insults.

Disclosures: S. Muscat: None. **N. Deems:** None. **M. Butler:** None. **R.M. Barrientos:** None.

Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 664.26

Topic: H.12. Aging and Development

Support: RF1AG028271
R03AG067061
R21AG071135

Title: The Effect of Short Term High Fat Diet on Pathology, Neuroinflammation, Synaptic Plasticity, and Phagocytosis in the 3xTg-AD Mouse Model

Authors: *S. MACKEY-ALFONSO[1], A. WILLIAMS-MEDINA[2], M. BUTLER[2], A. TAYLOR[2], N. DEEMS[1], R. M. BARRIENTOS[3];
[1]Neurosci. Grad. Program, [3]The Ohio State Univ., [2]The Ohio State Univ., Columbus, OH

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disease characterized by memory impairments and the hallmark pathology of neurofibrillary tau tangles and amyloid beta (Aβ) plaques. High fat diet (HFD) consumption has been shown to increase the risk of developing AD in humans and to cause memory impairments in AD mouse models. Previous research in the Barrientos lab has shown short-term HFD can cause neuroinflammation and memory impairment in wild type rat models. However, the underlying mechanisms linking diet to AD risk is not well-studied. Thus, this project serves to investigate the effect of HFD consumption on Aim 1) the severity of AD pathology in the hippocampus in a 3xTg-AD mouse model, Aim 2) neuroinflammation and synaptic plasticity genes in the hippocampus, and Aim 3) *In vitro* analysis of the effect of HFD on microglial phagocytosis of Aβ and synapses. Following the consumption of standard chow or 3 days of HFD, mice were saline-perfused, and the brains post-fixed in 4% paraformaldehyde and processed for immunohistochemistry (DAB) staining of phosphorylated tau (AT8) and Aβstaining in the CA1 region of the hippocampus. In a separate cohort, mice (5-8 month-old) were transcardially saline-perfused and the hippocampus was dissected for qPCR analysis of inflammatory and synaptic plasticity genes. In the *in vitro* study, synapses were isolated from mouse hippocampus. The isolated synapses and Aβ-42 were linked to pH-rhodamine which turns red when it is phagocytosed by cells. BV2 microglial cells were pretreated for 16 hours with palmitic acid, an important component of HFD, and uptake of pH-rhodamine linked Aβ, aggregated Aβ, and synapses was observed under a light microscope at 15-minute, 30 minute, 1 hour, 2 hour, and 4 hour time points. Photos were taken and quantified via ImageJ. Our DAB pathology data show HFD consumption significantly increased the number and size of AT8 and Aβ puncta in the hippocampus of 3xTg-AD mice. Short-term HFD significantly increased the level of proinflammatory markers and caused an alteration in synaptic plasticity genes in the hippocampus. The *in vitro* data show pretreated cells had decreased phagocytosis of Aβ, but increased uptake of synapses. These data suggest HFD consumption accelerates AD pathology, increases neuroinflammation, alters synaptic plasticity genes in 3xTg-AD mice, and leads to dysregulated microglial phagocytosis. Future studies will investigate the impact of HFD on learning and memory in AD mice and the cellular mechanisms driving these diet-induced inflammatory and pathological changes.

Disclosures: S. Mackey-Alfonso: None. **A. Williams-Medina:** None. **M. Butler:** None. **A. Taylor:** None. **N. Deems:** None. **R.M. Barrientos:** None.

Poster

664. Learning and Memory in Aging

Location: SDCC Halls B-H

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Program #/Poster #: 664.27

Topic: H.12. Aging and Development

Support: RF1AG028271
R21AG071135
R03AG067061

Title: Cd8+ t cells contribute to high fat diet-induced memory deficits in aged rats

Authors: *M. J. BUTLER[1], S. SENGUPTA[2], S. MUSCAT[2], N. DEEMS[3], S. A. AMICI[2], P. DRAVID[5], A. KAPOOR[5], M. GUERAU[2], R. M. BARRIENTOS[4];
[1]Ohio State Univ., [2]Ohio State Univ., Columbus, OH; [3]Ohio State Univ., [4]The Ohio State Univ., The Ohio State Univ., Columbus, OH; [5]Nationwide Children's Hosp., Columbus, OH

Abstract: Our lab has shown that short-term (3d) high fat diet (HFD) consumption induces a proinflammatory response in the hippocampus and subsequent impairment in long-term memory in aged, but not young adult, rats. However, the immune cell phenotypes driving this proinflammatory response are not well understood. We have shown that microglia from young and aged rats fed a HFD express similar levels of priming and proinflammatory transcripts, suggesting that factors independent of microglia may drive the exaggerated neuroinflammatory response in aged-HFD rats. T cells, which are known regulators of innate immune cells, infiltrate both the young and especially the aged CNS and contribute to immune surveillance of the parenchyma. Thus, we investigated whether short-term HFD in aged rats further increases T cell presence in the CNS and their role in learning and memory. Following 3d of HFD, young and aged rats were saline-perfused and brains were collected for qPCR or flow cytometry analyses. qPCR revealed alterations in T cell-specific transcripts in aged, HFD-fed rats. Flow cytometry indicated that aging alone increased total CD3+ T cells in the brain and that this effect was further increased in aged rats fed a HFD. CD4+ T cells did not account for this selective effect as increases were only observed as a function of aging, regardless of diet condition. In contrast, CD8+ T cells were selectively increased in aged, HFD-fed rats, suggesting they may play an important role in behavior. To determine the role of CD8+ T cells in cognitive function, we depleted circulating CD8+ T cells via an intravenous injection of an anti-CD8 antibody in aged rats prior to 3d of HFD. Rats then underwent contextual fear conditioning to test for long-term memory function. Results indicate that peripheral depletion of CD8+ T cells robustly prevented HFD-induced memory impairment in aged rats. Together, these data suggest a substantial role for T cells, specifically CD8+ T cells, in mediating aging + diet-induced memory impairments in male rats.

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Poster

664. Learning and Memory in Aging

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Program #/Poster #: 664.28

Topic: H.12. Aging and Development

Support: NIH Grant AG028271
NIH Grant AG067061
NIH Grant AG071135
NIH Grant AG028271-12S1

Title: Postoperative cognitive dysfunction: Long-term effects on neuronal structure and function

Authors: N. P. DEEMS[1], E. A. SCARIA[1], M. J. BUTLER[1], S. M. MUSCAT[1], B. M. GONZALEZ OLMO[1], T. T. QUACH[1], S. F. MAIER[5], G. P. CORTESE[6], ***R. M. BARRIENTOS**[1,2,3,4];
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Abstract: Post-operative cognitive dysfunction (POCD) is an abrupt decline in memory and executive function occurring in ~10% of older adult patients and lasting weeks to months after surgery, significantly increasing vulnerability to neurodegenerative diseases such as Alzheimer's disease. Our recently developed preclinical model of POCD determined that aged rats treated with the opioid, morphine, after abdominal surgery extended memory impairments from four days (without morphine) to eight weeks. The mechanisms driving opioid-exacerbated POCD are not well-understood, although significant increases in hippocampal-inflammatory gene expression two-weeks after surgery plays a key role. Given that hippocampal neurons and synapses are responsive to inflammatory signaling, we hypothesized that the combination of aging, surgery, and morphine, would alter structural and functional features of dendrites, dendritic spines, and synapses on CA1 neurons four-weeks after surgery. We used Golgi-Cox staining and reconstructions of CA1 pyramidal cells from the dorsal hippocampus to characterize dendritic complexity and length, and spine density and frequency of spine subtypes. We also collected hippocampal synaptoneurosomes to assess expression levels of plasticity-related proteins (PRPs) two-hours after a learning event, and measured LTP to identify any synaptic memory consolidation-related disturbances. Sholl analyses of CA1 dendrites revealed no structural changes in any group. Preliminary data indicate filopodia and mushroom spine density is decreased in aged, morphine-treated rats. Western blot analysis from hippocampal synaptoneurosomes showed a morphine-induced decrease of PRPs at baseline and an aberrant increase following a learning event, relative to controls. LTP data will be discussed. Taken together, these data suggest the combination of aging, surgery, and morphine disrupts dendritic spine density and proper balance of synaptic proteins necessary for memory consolidation.

Disclosures: **N.P. Deems:** None. **E.A. Scaria:** None. **M.J. Butler:** None. **S.M. Muscat:** None. **B.M. Gonzalez Olmo:** None. **T.T. Quach:** None. **S.F. Maier:** None. **G.P. Cortese:** None. **R.M. Barrientos:** None.

Poster

665. Probing Technologies I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.01

Topic: I.04. Physiological Methods

Support: NSF Grant GR37473

Title: Integrated fluorescence-guided micropipette for automated subtype-specific targeting

Authors: *E. B. MARSCHALL[1], J. F. LUSK[1], C. ARIDI[1], C. MIRANDA[2], B. S. SMITH[1]; [1]Arizona State Univ., Tempe, AZ; [2]Stanford Univ., Stanford, CA

Abstract: Patch-clamp electrophysiology is the gold standard for high-resolution recording of excitable cells. To achieve real-time subtype-specific targeting, image-guided systems have been developed which leverage fluorescent labels and dyes through high-powered microscopy systems (e.g., confocal and 2-photon)[1]. However, no such targeting method exists for specific subtypes beyond a tissue depth of 2mm due to the scattering of light[2]. In order to circumvent this limitation, waveguides have been used for controlled light delivery in deep tissue for applications including imaging, optogenetics, and photometry[3,4]. Our lab recently applied integrated waveguide technology wherein a traditional patch-clamping micropipette and tapered fiber optic were concentrically aligned to localize the fluorescence at the tip of the micropipette[5,6]. This method integrates waveguides into micropipettes, providing micropipettes with the ability to target fluorescence, and enabling high-resolution subtype-specific recordings of cells beyond 1mm. In this study, we precisely target and approach B35 neuroblastoma cells stained with Hoechst 33342 through our microscope-free technology. Introducing automated navigation capability into micropipette electrodes will allow precise targeting in combination with high resolution recording of cells at depths beyond what is currently possible.[1]Suk, H. J., van Welie, I., Kodandaramaiah, S. B., Allen, B., Forest, C. R., & Boyden, E. S. (2017). Closed-loop real-time imaging enables fully automated cell-targeted patch-clamp neural recording in vivo. *Neuron*, *95*(5), 1037-1047.[2] Xia, F., Gevers, M., Fognini, A., Mok, A. T., Li, B., Akabri, N., ... & Xu, C. (2022, May). Deep confocal fluorescence microscopy with single-photon superconducting nanowire detector. In *Advanced Photon Counting Techniques XVI* (Vol. 12089, pp. 25-29). SPIE.[3] Turtaev, S., Leite, I. T., Altwegg-Boussac, T., Pakan, J. M., Rochefort, N. L., & Čižmár, T. (2018). High-fidelity multimode fibre-based endoscopy for deep brain in vivo imaging. *Light: Science & Applications*, *7*(1), 1-8.[4] Miyamoto, D., & Murayama, M. (2016). The fiber-optic imaging and manipulation of neural activity during animal behavior. *Neuroscience Research*, *103*, 1-9.[5]Miranda, C., Marschall, E., Browning, B., & Smith, B. S. (2020). Side-viewing photoacoustic waveguide endoscopy. *Photoacoustics*, *19*, 100167.[6]Miranda, C., Howell, M. R., Lusk, J. F., Marschall, E., Eshima, J., Anderson, T., & Smith, B. S. (2021). Automated microscope-independent fluorescence-guided micropipette. *Biomedical Optics Express*, *12*(8), 4689-4699.

Disclosures: E.B. Marschall: None. **J.F. Lusk:** None. **C. Aridi:** None. **C. Miranda:** None. **B.S. Smith:** None.

Poster

665. Probing Technologies I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.02

Topic: I.04. Physiological Methods

Support: NIH Grant RF1MH117055

Title: Genetically Encoded Electrophysiology: Covalently Linking Neurons to Electrodes

Authors: *I. A. WEAVER[1], A. W. LI[1], B. C. SHIELDS[1], A. YOUSEFZADEH[1], S. S. X. LIM[1], V. GOLDENSHTEIN[1], P. P. VAGADIA[3], G. E. SCHILTZ[3], M. R. TADROSS[2]; [1]Duke Univ., [2]Duke Box 90281, 311 Res. Dr., Duke Univ., Durham, NC; [3]Ctr. for Mol. Innovation and Drug Discovery, Northwestern Univ., Evanston, IL

Abstract: Understanding how neural circuits encode behaviorally relevant information will require the ability to record from thousands of neurons simultaneously, with millisecond temporal precision, stability over many days, and knowledge of the genetic identity of each cell. Electrical recordings excel with regard to temporal fidelity, whereas optical tools have provided unsurpassed cell type specificity. Here, we describe a hybrid approach that seeks to combine the benefits of these two approaches. We engineered a conductive polymer incorporating a unique capture moiety, which can form a covalent bond to a genetically encoded protein. The conductive polymer can be grown on standard electrodes, while the genetic construct can be expressed on the surface of any neuron using standard AAV viral vectors. Preliminary data indicates that neurons expressing the genetically encoded protein can attach to the conductive polymer, as evidenced by their unique electrophysiological signature. In particular, detected spikes from a subset of these neurons (i.e. putative bound neurons) exhibit unusually large amplitudes, 200% - 500% the maximum amplitude seen from neurons expressing a control protein (i.e., unbound neurons). These data provide a necessary foundation for the development of genetically encoded electrophysiology, with potential to offer cell type-specificity, stability, millisecond temporal resolution, and scalability.

Disclosures: I.A. Weaver: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Pending, Duke University. **A.W. Li:** None. **B.C. Shields:** None. **A. Yousefzadeh:** None. **S.S.X. Lim:** None. **V. Goldenshtein:** None. **P.P. Vagadia:** None. **G.E. Schiltz:** None. **M.R. Tadross:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Pending, Duke University.

Poster

665. Probing Technologies I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.03

Topic: I.04. Physiological Methods

Support: NSF-I/UCRC award (#1650566)

Title: Assessing GFAP as an inflammatory biomarker in peripheral blood monocytes in response to chronic brain implants

Authors: *V. VOZIYANOV[1], A. SRIDHARAN[1], V. WHITE[2], U. MÜLLER[2], J. MUTHUSWAMY[1];
[1]Arizona State Univ., Tempe, AZ; [2]ZelosDX Inc., Tucson, AZ

Abstract: Immunohistochemical techniques are commonly used for characterizing the inflammatory response in the brain tissue due to the presence of microscale implants. Since immunohistochemistry is a terminal procedure unsuited for longitudinal experiments or clinical translation, we sought to employ a blood-based biomarker *in lieu* of immunohistochemistry. Such an approach would be minimally invasive, enabling chronic longitudinal studies and clinical translation. Based on our exploratory experiments we report a novel method for detecting trace amounts of brain-specific inflammatory proteins, such as glial fibrillary acidic protein (GFAP), from rodent blood draws. This method samples peripheral blood monocytes (PBMCs), which were recruited to the brain to aid the clearing of necrotic tissue debris, resulting from brain surgery and the implanted neural interface, and then re-enter the bloodstream. PBMC extracts can be tested for their biomarker content by ELISA, and the specific phagocytes involved can be identified and counted by flow cytometry. Our chronic experiments with male Sprague-Dawley rats had three cohorts: 1. Surgery, craniotomy, no implant (n=6), 2. Surgery, craniotomy, implant, no stimulation (n=5), and 3. Surgery, craniotomy, implant, and electrical stimulation (n=5). Blood was drawn from the saphenous vein two weeks before surgery, and 72 hours, 2, 4, and 6 weeks after surgery. For flow cytometry PBMCs were stained with fluorescently labeled antibodies to cell surface markers as well as GFAP, and extracts were tested by single-sided ELISA for their GFAP levels. We also made electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) measurements for the first four weeks during which electrical stimulation was performed. The results from these experiments show a significant increase in GFAP expression at 2 and 4 weeks compared to baseline, followed by a significant decrease in GFAP expression at six weeks compared to values at 2 and 4 weeks. These results are consistent with previous immunohistochemical studies that showed a similar peak in GFAP expression at those time points. In addition, flow cytometry analysis showed a similar increase in $CD43^{+}$ monocytes expressing GFAP at 2 and 4 weeks compared to baseline, which decreased at six weeks compared to values at 2 and 4 weeks. In conclusion, these experiments show that the novel blood-based assay can detect trace amounts of GFAP in PBMCs in response to microelectrode implantation and stimulation in chronic rodent experiments.

Disclosures: **V. Voziyanov:** None. **A. Sridharan:** None. **V. White:** A. Employment/Salary (full or part-time):; ZelosDX Inc. **U. Müller:** A. Employment/Salary (full or part-time):; ZelosDX Inc.. **J. Muthuswamy:** None.

Poster

665. Probing Technologies I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.04

Topic: I.04. Physiological Methods

Support: NSF Award No. 1728497
NIH Award No. NBIB DP2-EB029757
Support from CINT Facility, managed by Triad National Security, LLC for the U.S. Department of Energy's NNSA, under contract 89233218CNA000001

Title: 1024-channel, ultra-sharp, individually addressable silicon-based nanowire arrays for natively recording intracellular activities from neuronal networks

Authors: *J. LEE[1], Y. TCHOE[1], R. LIU[3], D. PRE[4], K. J. TONSFELDT[5], A. BOURHIS[1], A. D'ANTONIO-CHRONOWSKA[2], G. A. ROBIN[4], S. LEE[1], Y. RO[1], M. PHIPPS[6], J. YOO[6], J. NOGAN[6], J. MARTINEZ[7], K. FRAZER[2], A. G. BANG[4], S. DAYEH[1];
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Abstract: Intracellular access with high spatiotemporal resolution and minimal invasiveness is imperative in understanding how neurons regulate and orchestrate network activity, and how such activity can be influenced by pharmacology or other interventional methods. The gold standard technique to achieve high-fidelity intracellular recordings involves patch clamp method, but it only permits short recording duration and can potentially damage cell health. Other research primarily focuses on vertical, nanoscale interfaces and often employ electroporation or optoporation to permeate the cell membrane and obtain intracellular potentials; however, such methods tend to lead to attenuation of recorded potentials. In this work, we report high-density, vertical, ultra-sharp, silicon-based nanowire arrays to allow long-term, native recordings of intracellular potentials without any electroporation treatment. Fabrication of these ultra-sharp tip nanowires were processed with techniques that provided scalability to 1024 channels to achieve simultaneous recordings over extended areas, with each single nanowire electrode individually addressed to each channel. We employed top-down etching and successive thermal oxidation on silicon substrate to realize sub-10 nm nanowires, which were subsequently coated with platinum for electrical connection. The nanowire arrays were passivated with a dual layer of silicon oxide and plasma-treated parylene-C to promote neuronal cell culture adhesion and prevent delamination of metal interconnects. Previously fabricated nanowires have shown stable amplitudes of intracellular potentials from 3D tissue-like networks of rat cortical neurons across long culture times up to 19 days *in vitro*. Modulation of electrophysiological activities are demonstrated clearly with pharmacological drugs to either stimulate or inhibit neuronal activities. We detected graded membrane potentials prior to recorded action potential from neuronal recordings, exhibiting the ability of our platform to record subthreshold potentials, and also observed synaptic network activities between neurons. Our experiments and simulations demonstrate that as the number of nanowires per channel decreased, the peak-to-peak amplitude of intracellular signals increased, revealing the importance of individual addressability. This study advances the way toward predictive, high-throughput, and low-cost electrophysiological drug screening platforms.

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Poster

665. Probing Technologies I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.05

Topic: I.04. Physiological Methods

Support: NIH 5R01NS116464
NIH T32 EB029365

Title: Optical Dura: A high density optical surface LED array for non-human primates

Authors: *I. KIMUKIN[1], T. BELLOIR[2], D. J. GRIGGS[3], E. TOWE[1], A. YAZDAN-SHAHMORAD[4], M. CHAMANZAR[5];
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Abstract: Light-based methods are now being used to stimulate non-human primate (NHP) brains through optogenetics and also for functional recording through activity-dependent optical tags. To deliver light to the neural tissue in NHPs non-invasively, large- area, high density optical probes are needed. We have developed novel surface neural interfaces called Smart Dura for neural stimulation and recording. The Smart Dura is intended to replace the native dura and serve as a chronic permanent port to the NHP brain. The Smart Dura consists of an optical layer with a high density of sub-millimeter size light emitting diodes (LED) and an electrical layer consisting of a high density of microfabricated electrodes all embedded in a thin transparent layer of polydimethylsiloxane (PDMS). In this presentation, we focus on the optical layer of the Smart Dura, which we call oDura.
We have implemented oDura on Polyimide using advanced manufacturing techniques developed for realizing flexible printed circuit boards (PCBs). Arbitrary arrangements of individual LEDs can be implemented formed to match the target locations of the brain. We have developed devices with used blue (470nm), yellow (590nm), orange (605nm) and red (635nm) LEDs on our devices. Two different designs of the devices each with a diameter of 17.5mm were fabricated. The first, consists of 32 LEDs, each with dimensions of 1.6 x 0.8 mm and a narrow output angle (60o). The second, consists of 62 more compact LEDs (1.0 x 0.5mm), but with a wider output beam angle (140o).
Each individual LED can be controlled independently using our custom-designed microcontroller circuitry. Any desired spatial pattern of optical stimulation can be applied to the LEDs (updated every in less than 0.5 µs duration) to match the target locations in the brain. Light can be pulsed with pulse widths as small narrow as 0.5 µs.

To mitigate the heating issue of LEDs reduced tissue heating, especially due to their limited low efficiency of LEDs, we have developed an active cooling mechanism system using a thermo-electric cooler (TEC) and a heatsink to pump heat away from LEDs. We have implemented a closed-loop PID controller in the microcontroller with the help of thermistors on the surface of the probe. In our tests, we have achieved 1oC temperature stability even at high drive currents needed to reach brain regions as deep as 1 mm. This cooling mechanism enables us to stimulate neurons without the risk of damaging the tissue due to heat. We have tested these devices on a macaque that expresses JAWS opsin in motor cortex. We will discuss the design and characterization of oDura as well as proof of concept in vivo experiments to inhibit neural activity using JAWS opsin in NHPs.

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Poster

665. Probing Technologies I

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Topic: I.04. Physiological Methods

Support: NIH Grant P30DA048742
NIH Grant RF1NS126044
McKnight Foundation

Title: Inverse kinematic control of probe trajectories in multi-site neural recording experiments

Authors: M. FELDKAMP[1], *J. HOPE[1], Z. VIAVATTINE[1], P.-H. CHENG[1], T. BECKERLE[1], N. KANE[2], P. WILEY[2], M. SANDERS[2], J. ZHANG[3], T. PENGO[5], S. KODANDARAMAIAH[1,3,4];
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Abstract: The advent of neural probes with hundreds of recording sites along the probe shank has enabled researchers to capture neural recordings in mice from multiple brain regions simultaneously. By inserting multiple such probes into the brain along different trajectories, near brain-wide recordings are possible. However, probe insertion is often performed using a manipulator with several degrees of freedom of movement, making multi-site probe trajectory planning and insertion a highly complex operation even for 2-4 simultaneously inserted probes. We have developed a probe alignment platform to assist researchers in planning and executing multi-site neural recording experiments. The platform uses a kinematic description of the probe alignment mechanics to calculate the trajectory for each probe to hit its designated brain region. Constraints on solutions are imposed by the craniotomy dimensions, the necessity to avoid collisions between probe hardware, and the workspace of the probe alignment mechanics. We

next developed 3D printed polymer skulls that can be customized with entry ports corresponding to each probe. When the polymer skulls are chronically implanted on mice subjects, anatomical landmarks are used to reference the coordinate system of the mouse's brain to the coordinate system of the probe alignment platform. We characterized the probe alignment accuracy by inserting 4 dummy probes dyed with CM-Dil into several different networks of brain regions and then analyzing the brain samples using X-CLARITY tissue clearing and ribbon scanning confocal microscopy for 3D imaging. We measured an average error of 0.37 +/- 0.20 mm, indicating the methodology can be used to effectively localize probes to most desired target regions of interest which are accessible through the dorsal cortex. We then demonstrated the platform's functionality by acquiring a series of acute bilateral neural recordings from the somatosensory and visual cortices across several days in a chronically implanted mouse. Lastly, an *in silico* analysis was performed that used a heuristic algorithm to determine the optimal probe configurations for any given set of brain regions and workspace. The results indicate our system should assist in planning and accomplishing complex multi-site neural recording experiments and in the future may be used to guide robotic neural probe implantation in humans.

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Poster

665. Probing Technologies I

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Topic: I.04. Physiological Methods

Support: NIH R01 Grant NS104344
Eugene McDermott Graduate Fellowship 202108
NIH R01 Grant NS110823

Title: Intracortical Microstimulation Behavioral Paradigm for the Evaluation of Stimulation-Evoked Somatosensory Percepts in Rodents

Authors: *T. J. SMITH[1], Y. WU[2], J. R. ABBOTT[2], J. R. CAPADONA[4], C. T. ENGINEER[3], S. F. COGAN[2], J. J. PANCRAZIO[2], A. G. HERNANDEZ-REYNOSO[2];
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Abstract: Intracortical microelectrode arrays (MEAs) are capable of evoking various percepts through intracortical microstimulation (ICMS) in patients with paralysis and sensory loss. However, a behavioral task for assessing somatosensation in animal models without the use of pain aversive techniques has yet to be demonstrated. Recently, our lab has developed a positive-reinforcement operant conditioning behavioral paradigm, based on the well-established nose-

poking task to quantify rodent perception thresholds in response to ICMS. Our goal is to develop a highly reproducible protocol for investigating the reliability of chronic ICMS and MEA longevity in-vivo. All procedures were approved by the University of Texas at Dallas IACUC. Sprague Dawley rats were first habituated and evaluated for proficiency in a nose-poking task prior to randomized assignment to either receive a) an implanted MEA device targeting the primary somatosensory cortex (S1FL) or b) no implant, acting as a negative control group. The ICMS group was trained in a custom operant conditioning apparatus (OmniTrak from Vulintus, Inc.) to nose-poke (conditioned response) following an ICMS square biphasic pulse wave (conditioned stimulus) to receive a sugar pellet (unconditioned stimulus) within a specified response window. The control group then paralleled the same behavioral task, but without receiving a conditioned ICMS stimulus. ICMS parameters were the same as previously established by other groups: charge balanced, biphasic waveform, 320 Hz frequency, 200 µs pulse width, 40 µs interphase interval, 650 ms train duration, 0-10 nC/phase charge (0-50 µA). A confusion matrix based on animal responses (True/False pokes vs. non-pokes) was generated and used to calculate accuracy, precision, sensitivity (recall), specificity and F1-score. Finally, chi-square tests were used to calculate significance responses within groups. Results over 291 trials showed that the ICMS animals had an accuracy of 74.9%, precision of 75.6%, sensitivity of 70.7%, specificity of 78.8%, and a F1-score of 73.1%. In contrast, the control group observed an accuracy of 48.0% over 273 trials, indicating random nose-poking and overall decrease in precision, sensitivity, specificity and F1-score (47.5%, 64.7%, 32.1%, and 54.8% respectively). The relation between responses for the ICMS group was statistically significant $p < 0.0001$, but not for the control group ($p=0.58$). These results suggest that receiving ICMS as the conditioned stimulus can significantly improve the reliability of a rat's ability to accurately nose-poke when prompted, validating this paradigm as a tool for future perception detection studies.

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Poster

665. Probing Technologies I

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Topic: I.04. Physiological Methods

Support: DFG (SPP 1665 FR2557/1-1, FOR 1847 FR2557/2-1, FR2557/5-1-CORNET, FR2557/7-1-DualStreams to P.F.)
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LOEWE program (NeFF to P.F.)

Title: Modular, cement-free, customized headpost and pedestal implants for macaques

Authors: *E. PSAROU[1,2], J. VEZOLI[1], M. L. SCHÖLVINCK[1], P.-A. FERRACCI[1], Y. ZHANG[1,2,3], I. GROTHE[1,4], R. ROESE[1], P. FRIES[1,2,3];
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Abstract: Neurophysiological studies with awake macaques require the use of chronic cranial implants that can stay healthy for an extended period of time. Headpost implants are widely used to provide head fixation during experimental procedures. We describe several refinements of the entire head fixation technique of macaque monkeys, including the implant itself, the surgical procedures and the everyday handling. We developed modular headposts made of titanium that consist of a baseplate and a top-part. These pieces are implanted in two surgeries separated in time by several weeks. The implant baseplate was customized to fit the individual animal's skull either by manual bending or computer numerical control (CNC) milling. We present a clamping approach that facilitated the precise milling of the thin footed baseplate, which can otherwise be a challenging procedure. In the first surgery, the baseplate is anchored onto the skull using merely titanium bone screws. The screw length is pre-operatively planned and adjusted to match the skull thickness. At the end of this surgery, the baseplate is covered with muscle and skin. The surgical site is closed and allowed to heal for several weeks. Isolating the implant from the external world reduces the risk of post-operative infection, thus promoting initial osseointegration. Following complete healing of the wound margin, the percutaneous top-part is added in a short surgery. A small area of the previously implanted baseplate is exposed using a special punch tool. This tool achieves a perfectly round skin cut that allows the implantation of the top-part and provides a tight fit of the skin around it without any sutures. This speeds up the implantation procedure and reduces the risk of post-operative complications that can arise from suture opening. To improve handling safety, we introduced a headpost holder that can be remotely attached to the headpost top-part to stabilize the animal's head. Twelve male monkeys were successfully implanted with the modular headpost. To date, there has been no implant failure or loosening from the bone, in some cases more than 9 years after top-part implantation. Finally, a modular, cement-free and footless pedestal implant for mounting a connector chamber was designed to achieve a minimized footprint on the skull and to allow for a similar two-step implantation approach. This pedestal was implanted in one macaque so far. We conclude that the use of modular implants is safe and provides increased flexibility because the top-part can be exchanged and modified. Modular implants also allow for a two-step implantation that can reduce the risk of post-operative complications and thus benefit animal welfare.

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Poster

665. Probing Technologies I

Location: SDCC Halls B-H

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Program #/Poster #: 665.09

Title: WITHDRAWN

Poster

665. Probing Technologies I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.10

Topic: I.04. Physiological Methods

Support: SPG Grant RG101412

Title: Biofouling performance of novel boron-doped diamond microelectrodes during dopamine detection using fast-scan cyclic voltammetry

Authors: *B. GUPTA[1], J. R. SIEGENTHALER[4], J. B. LANDGRAF[5], M. PERILLO[2], R. RECHENBERG[6], M. F. BECKER[4], W. LI[3], E. K. PURCELL[2];
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Abstract: While carbon fiber microelectrodes (CFMEs) have long been a standard technology for neurochemical sensing, boron-doped diamond (BDD) is an emerging material with promising properties for analytical measurements. BDD electrodes offer wide working potential windows, biocompatibility, low electrochemical background currents and stability. Recently, our team has developed an all-diamond BDD microelectrode (BDDME), with a conductive boron-doped diamond core, and an insulating polycrystalline diamond shell, for the detection of dopamine (DA) and other neurotransmitters relevant to neurological diseases. An important potential feature of the BDD is resistance to biofouling; an issue faced by prolonged implantation of electrodes in the brain resulting in diminished neurochemical sensing. In this study, we sought to characterize the *in vitro* biofouling performance of the novel BDDME. Using fast-scan cyclic voltammetry (FSCV) with a BDDME, we measured dopamine in a flow-injection-cell to compare the electrode response both pre- and post-exposure to both brain tissue homogenates and a commonly used biofouling agent, bovine serum albumin (40 g L^{-1}, in artificial cerebrospinal fluid, pH 7.4). CFMEs were also tested to serve as a baseline comparison for the BDDMEs. Additionally, different surface modifications, including PEDOT:Nafion coatings, known to reduce biofouling, were tested on both the BDDMEs and CFMEs to assess improved sensitivity to neurochemicals compared to the non-coated electrodes. Linear calibration curves of the electrode responses to a range of dopamine concentrations were constructed for each

electrode before and after biofouling. Similarly, background currents of each electrode were compared both pre- and post-fouling. This study is foundational to understand how different surface modifications and/or fabrication schemes change the biofouling performance of the BDDME. These results obtained here are an important step towards the *in vivo* use of the BDDME for DA detection, which we are currently assessing.

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Poster

665. Probing Technologies I

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Topic: I.04. Physiological Methods

Support: NIH Grant R01NS116080

Title: Optimizing all diamond boron doped diamond microelectrodes for neurotransmitter measurement with fast-scan cyclic voltammetry

Authors: *J. R. SIEGENTHALER[1], J. B. LANDGRAF[1], B. GUPTA[2], R. RECHENBERG[1], G. M. H. U. BANNA[3], D. GALSTYAN[1], A. HARDY[1], M. F. BECKER[1], E. K. PURCELL[3], W. LI[3];
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Abstract: Diamond is a versatile material that has good biocompatibility, is chemically inert and when doped to be conductive, has a wide working potential window in aqueous solutions for electrochemical detection. Boron-doped diamond microelectrodes have been previously grown on sharpened tungsten wire and used to measure dopamine and other electroactive neurotransmitters both *in vitro* and *in vivo* with high spatial and temporal resolution using fast-scan cyclic voltammetry (FSCV). Here we report on the further development and optimization of boron-doped diamond electrodes to feature an all diamond microelectrode grown as a solid core that can electrochemically measure common neurotransmitters using FSCV showcasing a linear dynamic response. The all-diamond microelectrode consists of a boron-doped polycrystalline diamond core encapsulated in an insulating polycrystalline diamond shell improving on the biocompatibility, and flexibility of traditionally hand fabricated microelectrodes. These boron-doped microelectrodes (BDDME) are rectangular in structure, with a cleaved planar tip for electrochemical sensing. Using these electrodes, we studied how a cleaved BDDME surface compared with a laser-cut BDDME surface and looked at surface enhancements and cleaning methods. Additionally, we characterized several common neurotransmitters for the linear dynamic range, detection limit, and noise measuring dopamine, serotonin, 3,4-Dihydroxyphenylacetic acid, ascorbic acid, and hydrogen peroxide using FSCV. Using all

diamond electrodes for neurotransmitter analysis is advantageous as it is the gateway to wafer batch fabrication of microelectrodes, decreasing errors generated in the traditional hand fabrication methods, and building towards a scalable batch method for electrode array technologies.

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Poster

665. Probing Technologies I

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Program #/Poster #: 665.12

Topic: I.04. Physiological Methods

Support: DA51100
AI145206

Title: Electrochemical aptamer-based sensors for exploring the relationship between in brain procaine concentrations and associated behavioral responses

Authors: *K. M. HONEYWELL, J. GERSON, M. K. ERDAL, M. H. MCDONOUGH, K. K. LEUNG, M. R. STOCCO, N. E. EMMONS, J. GIBSON, J. P. HESPANHA, W. MEIRING, K. W. PLAXCO, T. E. KIPPIN;
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Abstract: Drug induced behavioral effects are concentration-dependent, and the current measurement techniques do not have sufficient temporal resolution to capture changes in drug concentration associated with physiological processes and thus behavioral effects. Procaine is a psychoactive compound with a short half-life and the ability to elicit behavioral effects following intravenous administration, making this drug our choice for determining the relationship between in brain concentrations and corresponding behaviors. We employed electrochemical aptamer-based (E-AB) sensors for the measurement of procaine *in vivo* in awake, freely behaving Sprague-Dawley rats. Our E-AB sensors have sufficient sensitivity, temporal resolution (~ 12 s), and stable drift characteristics to resolve the pharmacokinetics of intravenously administered procaine in the lateral ventricle of the brain. These data are combined with standard locomotor measurements to enable detailed analyses of the relationship between in brain concentrations and behavioral responses for individual male and female subjects. Additionally, we employed these E-AB sensors to support feedback-controlled delivery of procaine to produce constant in brain drug concentrations over time while taking into account individual differences in pharmacokinetics. As procaine concentrations increased, locomotor activity decreased and, in the case of feedback control, remained low at controlled procaine levels. In conclusion, our E-AB sensor platform can determine in brain pharmacokinetics of psychoactive drugs including

procaine in behaving animals, which can enable the exploration of the drug concentration-behavioral response relationship and the maintenance of specific drug concentrations via feedback controlled drug delivery.

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Poster

665. Probing Technologies I

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Program #/Poster #: 665.13

Topic: I.04. Physiological Methods

Support: Joint research with Ushio, Inc.

Title: Development of a microfluidic culture device for in vitro neural toxicity assessment with AI image analysis

Authors: X. HAN[1], N. MATSUDA[1], M. YAMANAKA[2], ***I. SUZUKI**[1];
[1]Tohoku Inst. of Technol., Tohoku Inst. of Technol., Sendai, Miyagi, Japan; [2]Ushio Inc., Yokohama, Japan

Abstract: Microphysiological system (MPS) is an in vitro culture technology that reproduces the physiological microenvironment and functionality of humans, and is expected to be applied for drug screening. In this study, we develop a culture device and related evaluation method using AI image analysis for the purpose of constructing a rapid assessment platform for peripheral neuropathy caused by compounds. First, we developed a microfluidic culture device that could separate the cell body and neurites, so that elongated neurites can be analyzed alone. In this device, the microchannel resin member is manufactured by direct photobonding (R), and there is no effect on the culture evaluation system due to the elution of the adhesive. COP (Cyclo olefin polymer), which has excellent observability and low drug adsorption, is used as the resin material. Next, dorsal root ganglion collected from E15 rat was cultured in the device coated with Poly-L-lysine and Laminin-511. After culturing, the neurites were elongated unidirectional along the microchannel. The morphological changes of neurites can be clearly analyzed by immunostaining. In addition, at 6th week of culture, myelination was confirmed from the axons extending in the channel. Successful culture of separated neurites in the microchannel device for more than 1 month indicates that a series of test processes from culture to drug stimulation and fluorescence observation is possible. Finally, we treated cultured neural samples with several anticancer drugs known to cause peripheral neurotoxicity (i.e., paclitaxel, vincristine, oxaliplatin, and suramin), and analyzed neurites morphological changes by AI machine learning. After

training, AI could identify neurite damage caused by each compound even at a quite low concentration. Therefore, this microfluidic culture system is supposed to be useful for in vitro toxicity assessment.

Disclosures: **X. Han:** None. **N. Matsuda:** None. **M. Yamanaka:** None. **I. Suzuki:** None.

Poster

665. Probing Technologies I

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Program #/Poster #: 665.14

Topic: I.04. Physiological Methods

Support: Institutional Funds

Title: Variation in platinum-iridium chronic depth electrode design decreases cortical injury and apoptosis in mouse brain.

Authors: ***B. HINTZE**[1], N. R. RENSING[2], L. HAN[2], T. J. FOUTZ[2];
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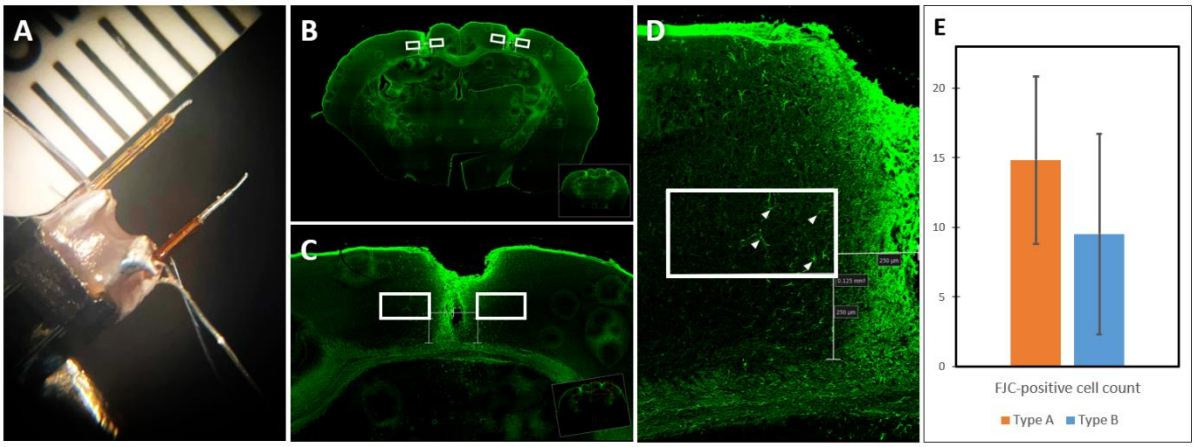
Abstract: Mitigating brain injury induced by chronic depth electrodes used for neurostimulation plays a crucial role in maintaining effective neurostimulation. The current study contrasts cortical damage caused by distinct depth electrode designs by quantifying the impact of implantation on tissue injury and cellular apoptosis. These effects were assessed in a mixed C57Bl/6 and SV129 genetic background mouse model. Two intracortical depth electrode designs were evaluated. The first design (Type A) used a bare platinum-iridium wire core surrounded by deactivated fused silica (DFS). The second design (Type B) was composed of a PTFE-coated platinum-iridium wire core, only partially surrounded by DFS. The DFS entered the substance of the brain in the type A design but not in the type B design. The completed head stage (Fig. A) included four stainless steel screw electrodes for recording EEG and one depth electrode of each design for stimulation. The head stages were surgically implanted, and the brains were harvested five days post-operatively. The implanted brains were then fixed with paraformaldehyde overnight and dehydrated with sucrose for a minimum of 24 hours. They were then sectioned coronally with a freezing microtome and collected for each mouse at the level of the hippocampus. Sections were stained for Fluoro-Jade C (FJC), mounted, and imaged using a Hamamatsu NanoZoomer 2.0 microscope (Fig. B, C). For each electrode, the FJC-positive cells were counted from two or more sections within bilateral 0.125 mm^2 areas, drawn symmetrically and equally spaced around the injury track (Fig. D). The type A design showed a mean of 9.5 (SD ± 6.0) apoptotic cells within the given area, whereas the type B design showed a mean of 14.8 (SD ± 7.2) (Fig. E). The p-value for a two-sample unequal variance t-test was 0.018 (n=5), demonstrating statistical significance. Thus, the type B design was associated with a significantly

lower rate of apoptosis and brain injury than the type A design. These results provide insight into the effects of different invasive EEG electrode designs on tissue and cellular injury.



Disclosures: **B. Hintze:** None. **N.R. Rensing:** None. **L. Han:** None. **T.J. Foutz:** None.

Poster

665. Probing Technologies I

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.15

Topic: I.04. Physiological Methods

Support: Israeli Ministry of Science and Technology: #315629 and #315240
Bar-Ilan President scholarship
Bar-Ilan Institute of Nanotechnology and Advanced Materials scholarship

Title: Controlling 3-dimensional neural network formation using magnetic manipulations

Authors: *R. PLEN[1], A. SMITH[1], U. LOCKER[1], Z. SHAPIRA[1], S. MARGEL[1], O. SHEFI[2];
[1]Bar-Ilan Univ., Ramat Gan, Israel; [2]Fac. of Engin., Ramat-Gan, Israel

Abstract: Controlling the motility and organization of nerve cells to form pre-designed 3D neural networks is essential for developing neuronal interfaces and new regeneration approaches. Due to the limited ability of damaged nerve cells to spontaneously repair and regain functionality physical trauma, restoring tissue integrity and functional capabilities have long been a major challenge in the field of tissue engineering. Here, we present a unique nano-based approach for dynamic localization of cells within 3D biomaterials using magnetic manipulations. We designed and fabricated magnetic arrays for exerting specified magnetic fields based on COMSOL simulations. We synthesized superparamagnetic nanoparticles (MNPs) for cell uptake and safe cell-MNP interactions. We transformed nerve cells (PC12 cell line and primary mice cortical cells) into magnetic units by incorporating them with the MNPs. We subjected the cells to multiple magnetic fields using the pre-designed magnetic arrays and manipulated their

organizational layout inside multi-layered 3D collagen scaffolds in a significant manner (p-val<0.0001, evaluated by one-way ANOVA followed by Tukey post-hoc test). Via these magnetic manipulations, we created functional 3D microarchitectures of neural networks demonstrating clustered and layered structures. This strategy paves the way to creating customized 3D tissue architectures for bioengineering applications and provides efficient models for investigating cellular and tissue behavior with a view toward a clinical approach.

Disclosures: R. Plen: None. **A. Smith:** None. **U. Locker:** None. **Z. Shapira:** None. **S. Margel:** None. **O. Shefi:** None.

Poster

665. Probing Technologies I

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 665.16

Topic: I.04. Physiological Methods

Support: CIHR FDN 143337

Title: A thermode for precise intrabrain temperature stimulation

Authors: *C. ZAELZER[1], C. BOURQUE[2];
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Abstract: A vital characteristic of mammals is their ability to maintain core body temperature (TBODY) near an ideal set point of ~37°C [1, 2]. The control of TBODY is mediated by an array of autonomic, endocrine and behavioral mechanisms supporting concerted osmo- and thermo-regulation [3, 4]. Previous work has shown that a collection of nuclei in the hypothalamus, known as the preoptic area (POA), contains neurons that are intrinsically thermosensitive [5, 6]. Local heating of the POA (which includes MnPO and OVLT areas) can evoke cooling responses (including ↓TBAT and ↑TTAIL), whereas local cooling evokes body warming (↑TBAT and ↓TTAIL [1, 2]). Despite the knowledge contributed in the last years, is still difficult to precisely pinpoint the location inside the POA that orchestrates those changes. This is crucial to answering questions concerning the molecular identity of the ion channels responsible for physiological thermosensation, the circuits that those organs innervate, and the role of local thermosensitivity of POA neurons under physiological conditions inside the brain.Here we introduce the design of a thermode constructed using coated copper and nichrome micro wire that acts as a heating element of ~ 0.4 mm wide by ~ 0.6 mm long and is mounted on the tip of a 26 gauge needle. The needle is glued to a magnetic connector that allows stereotaxic implantation on the mouse brain and controls up to +5°C from basal temperatures over a precise area.Calibration of the thermode at room temperature, 37°C, and 37°C warm water shows a precise and restricted area for thermal stimulation delivery (0.4 x 0.6 mm) and a negative relationship between temperature and distance from the stimulation source.Infrared imaging of freely-moving mice was then used to examine the effects of local heat delivered to the OVLT/ventral MnPO. Increasing temperature

by +2°C induced a dramatic increase in tail temperature from ~4°C to ~30°C. These changes were accompanied by a decrease in BAT temperature (~38°C to ~36°C) and eye temperature (used to monitor body temperature) (~36°C to ~34°C).

Disclosures: C. Zaelzer: None. **C. Bourque:** None.

Poster

665. Probing Technologies I

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Program #/Poster #: 665.17

Topic: I.04. Physiological Methods

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National Natural Science Foundation of China Outstanding Young Researcher Award (grant no. 30525016)
Project 985 grant of Peking University, Beijing Municipal Commission of Science and Technology (grant no. Z18110000151)

Title: A large calcium imaging dataset reveals natural image feature map in macaque V4

Authors: *T. WANG, H. YAO, S. TANG;
Peking-Tsinghua Ctr. for Life Sci., Beijing, China

Abstract: Understanding how diverse visual inputs in the real world are represented by brain requires sampling neural population responses to large number of stimuli. Here we present a widefield calcium imaging dataset that includes V4 cortical responses to over 17K natural scenes in three macaque monkeys. The dataset provides columnar scale neural population responses with a high signal-to-noise ratio. To extract the encoding law contained in the data, we used the dataset to train deep neural network models that predict cortical response more accurately than state-of-the-art models from computer vision. These high accuracy models revealed comprehensive maps of imaged V4 areas that contain various functional domains preferring from orientation, color, and curvature, to spot, grid, and face components. The model predicted maps were further verified by widefield imaging and single-cell resolution two-photon imaging. These results show that the large-scale calcium imaging dataset provides a valuable source for systematically understanding neural coding of visual cortex.

Disclosures: T. Wang: None. **H. Yao:** None. **S. Tang:** None.

Poster

666. Probing Technologies II

Location: SDCC Halls B-H

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Program #/Poster #: 666.01

Topic: I.04. Physiological Methods

Support: H2020-MSCA-IF-2019 grant 896996

Title: Toward CMOS neural probes with micro-wire like cross-sectional sizes: the ChroMOS probes

Authors: *J. F. RIBEIRO[1], A. PERNA[1,2], G. ORBAN[1], L. GIANTOMASI[1], M. VINCENZI[1], F. BOI[1], G. N. ANGOTZI[1], L. BERDONDINI[1];
[1]Italian Inst. of Technol., Italian Inst. of Technol., Genova, Italy; [2]The Open Univ. Affiliated Res. Ctr. at Inst. Italiano di Tecnologia (ARC@IIT), Genova, Italy

Abstract: The performance in chronic conditions of intracortical implantable multielectrode array probes is a key factor to enable studying how assemblies of individual neurons implement and execute brain functions and how they are hacked in brain diseases. Achieving such chronic stability is an important challenge in the field of neurotechnology. A successful emerging approach to mitigate brain tissue inflammatory responses to the implant consists in the development of polymeric neural probes featuring small cross-sectional sizes. However, the number of microelectrodes that can be integrated on such probes is severely limited by the necessity of individually wiring each electrode through the shank until the base of the probe. Alternatively, active dense implantable neural probes based on CMOS technology, such as Neuropixels, Neuroseeker or, more recently, the SiNAPS-probes developed in our lab, might allow overcoming such limitation in the number of integrated electrodes over small cross-sectional shank sizes. In particular, differently than other CMOS neural probes that integrate low noise neural amplifiers in the probe base, the SiNAPS technology allows to avoid most of the connection lines in the shanks by using in-pixel amplifiers integrated beneath each electrode and multiplexing circuits to reduce the number of output lines. However, it remains unclear whether such Si based CMOS devices can be effective in chronic intracortical interfacing. Here, we report the first results obtained on the development of micro-wire like ChroMOS probes based on the SiNAPS technology. Such ChroMOS probes feature a shank 3.9 mm long, 26 µm to 30 µm wide and 15 to 20 µm thick, and integrate 8 modules of 8 electrodes/pixels each (64 electrodes) distributed in a single column. The 10 µm diameter Pt electrodes have a pitch of 29.5 µm in the same module and 89.7 µm pitch between modules (active area of 2.3 mm). Bench top and in-vivo preliminary results obtained with these ChroMOS probes will be presented and discussed.

Disclosures: **J.F. Ribeiro:** None. **A. Perna:** None. **G. Orban:** None. **L. Giantomasi:** None. **M. Vincenzi:** None. **F. Boi:** None. **G.N. Angotzi:** None. **L. Berdondini:** None.

Poster

666. Probing Technologies II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 666.02

Topic: I.04. Physiological Methods

Title: Sinaps: a flexible and scalable cmos based neural probe technology for high-density full-band extracellular brain mapping

Authors: *F. BOI[1], G. N. ANGOTZI[2], J. F. RIBEIRO[3], M. VINCENZI[4], G. ORBAN[5], A. LOCARNO[6], R. TONINI[8], L. BERDONDINI[7];
[1]Inst. Italiano di Tecnologia, Genova, Italy; [2]Inst. Italiano Di Tecnologia, Genova, Italy; [3]NetS3, Italian Inst. of Technol., Genova, Italy; [4]Microtechnology for Neuroelectronics (NetS3), Fondazione Inst. Italiano di Tecnologia, Genova, Italy; [5]Fondazione Inst. Italiano di TEcnologia, Genova, Italy; [6]Neuromodulation of Cortical and Subcortical Circuits Lab., [7]Neurosci. and Brain Technologies, Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [8]Neuromodulation of Cortical and Subcortical Circuits Lab., Fondazione Inst. Italiano Di Tecnologia, Genova, Italy

Abstract: Active dense CMOS neural probes for extracellular recordings enable electrophysiologist to dissect, with an unprecedented spatio-temporal resolution, brain dynamics from the network scale down to single cells. Unlike other proposed CMOS-probes, based on AC-coupled frontend amplifiers connected through a switching matrix to the recording sites, the SiNAPS technology developed in our laboratory is based on a radically different DC-coupling approach. In particular, the scaling issue of analogue front ends is overcome by integrating these circuits underneath each electrode pixel site. Thanks to the high-modularity of the electrode-pixels-based SiNAPS technology, we have realized several different layouts adapted to different animal models and with increasing number of simultaneously recording electrode channels. Here we will report the state of the art of the SiNAPS probes realized so far (from 256 up to 1024 simultaneously recording channels) and their application to different animal models, with a particular focus on electrophysiological brain mapping and coupling with opto-stimulation. Experimental data will report the reached performances of these devices for the acquisition of full-band neural signals (LFPs and spikes) sampled at 20kHz, with a gain of 46dB, an input referred noise in the action potential band (300-7000 Hz) is of 6.5 ± 2.1μVRMS, and with a power consumption <6 μW. Finally, we will present and discuss a SiNAPS probe prototype suitable for deep brain recordings in non-human primates. This device allows resolving the activity of hundreds of different single-neurons and paves the way to new discoveries and knowledge useful for translational applications towards the clinical field.

Disclosures: F. Boi: None. **G.N. Angotzi:** None. **J.F. Ribeiro:** None. **M. Vincenzi:** None. **G. Orban:** None. **A. Locarno:** None. **R. Tonini:** None. **L. Berdondini:** None.

Poster

666. Probing Technologies II

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Program #/Poster #: 666.03

Topic: I.04. Physiological Methods

Support: NIH grant R01NS123069

Title: Bundle of Thin Multifunctional Fibers Enables Multi-colors, Multi-drugs, and Multi-site Recording

Authors: *J. KIM[1], E. GILBERT[2], H. HUANG[1], D. F. ENGLISH[2], X. JIA[1];
[1]The Bradley Dept. of Electrical and Computer Engin., [2]Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: A fiber-based multifunctional probe is an excellent interfacing platform for simultaneous electrical recordings, optical stimulations, and drug deliveries within the deep brain. However, due to the complexity and challenges at the back-end connections of each probe, the quantities of these modalities are restricted. Here, we present a novel method of increasing the number of electrodes, optical waveguides, and microfluidic channels in our newly designed probes by fabricating smaller and denser fibers and bundling them in a custom thermally drawn scaffold. Using the convergence thermal drawing method, we draw a fiber (D: 50μm) with 15μm tungsten electrodes, an optical waveguide, and/or a microfluidic channel. Then, the bundling system allows for customizing the final neural probes, allowing easier connections of the various modalities. We demonstrated various designs of the customized fiber-based probes in the wild-type mice (C57BL6/J x FVB/NJ). Single unit cells, sharp-wave ripples, and the prominent theta waves are recorded at the pyramidal cell layer in CA1. Six fiber probes (24 electrodes) are attached to a customized microdrive enabling chronic bilateral recordings and stimulations in the hippocampus. The neural responses to the simultaneous 450nm and 635nm optical stimulation are recorded to demonstrate the optical waveguides. To demonstrate the drug delivery channel, we locally injected cannabinoid into CA1 and observed changes in the neural activities. These new bundled fiber-based probes have increased numbers of electrodes, optical waveguides and microfluidic channels while reducing the tissue damages/imprints.

Disclosures: J. Kim: None. **E. Gilbert:** None. **H. Huang:** None. **D.F. English:** None. **X. Jia:** None.

Poster

666. Probing Technologies II

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Program #/Poster #: 666.04

Topic: I.04. Physiological Methods

Support: NIH grant R01NS123069

Title: Scalable Thermally Drawn Multifuctional Fiber-based Neural Interfaces Enabled by Backend Connection

Authors: *H. HUANG[1], J. KIM[1], E. GILBERT[1], D. F. ENGLISH[2], X. JIA[1];
[2]Neurosci., [1]Virginia Tech., Blacksburg, VA

Abstract: Neural probes provide valuable insight to the nervous system, helping advance medicine and biotechnologies. Thermally drawn fiber-based probes offer a cheaper and more biocompatible alternative to traditional silicon counterparts. However, the current fabrication and connection process limits the scalability, feature density, size, and geometry of these devices. To overcome such limitations and explore new fiber designs, we developed a novel fabrication and connection process that allows rapid scalable backend connection of any number of features distributed in an arbitrary geometry, increasing the competitiveness of thermally drawn neural probes. This method was then utilized to fabricate and fully connect several multi-functional neural probes of different configurations that were not possible to connect with existing methods. These include, e.g. (1) a 4 waveguide, 4 microfluidic channel, and 8 electrode probe; (2) an 8 waveguide and 8 microfluidic probe; and (3) a 16 microfluidic channel drug delivery system. Each electrode, waveguide, and microfluidic channel is individually addressable. To make the devices, large diameter fibers are thermally drawn and tapered, yielding big backends for connection and small sensing ends for minimally invasive implantation. All connection processes are then accomplished via backend connection. One of the fabricated probes was implanted into transgenic mice. Neural signals were recorded, and optical stimulation performed, demonstrating the device's optical and electrical capabilities. Drugs were injected via the probe's microfluidic channel, and their effects on recorded neural signals detected. Finally, chronic implantations were conducted on mice, validating the device's high biocompatibility. Our experiments demonstrate that the new fabrication and connection process for thermally drawn fibers have successfully overcome numerous limitations of the original process, paving the way for future probes of greater complexity and functionality.

Disclosures: **H. Huang:** None. **J. Kim:** None. **E. Gilbert:** None. **D.F. English:** None. **X. Jia:** None.

Poster

666. Probing Technologies II

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 666.05

Topic: I.04. Physiological Methods

Title: Self-assembled stacked flexible neural probes for scalable and highly versatile neural interfacing

Authors: *D. YAN, D. JEONG, J. R. LOPEZ RUIZ, E. YOON;
Electrical Engin. & Computer Sci., Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Channel-count scaling and multi-function integration have been two of the essential prerequisites for the next-generation neural probes. Efforts have been made to achieve these goals by monolithically integrating more electrodes and adding functional components on the same substrate. However, the complexity, cost, and yield of microfabrication often do not scale linearly and also limit versatility. In this work, we present an approach to combine multiple thin polyimide flexible neural probes of different functionalities in hybrid self-assembly as a cost-effective way to increase the channel counts and provide multi-functionality. Each individual neural probes were fabricated separately and embedded with an array of $Ni_{80}Fe_{20}$ micro-magnets. By using these micro-magnets with specific patterns, individual neural probes could be self-assembled in liquid with an external magnetic field applied. A pair of the probes could be self-assembled in less than 1 minute with a misalignment of less than 20-μm in longitudinal direction and 5-μm in transverse direction. As a proof of concept, we built a high-density flexible probe with 128 recording sites and 3 dopamine sensing electrodes by stacking three individual probes in a self-assembly manner. The dopamine sensing electrodes were made of surface roughened Pt coated with graphene oxide for enhanced sensitivity. The detection limit and sensitivity of the dopamine sensing electrodes were 1 μM and 0.2 nA/μM, respectively. The device preparation, *in vitro* characterization and *in vivo* validations are presented. This innovative approach to extend the number of channels and functionality can provide scalable and highly versatile neural probes for comprehensive analysis of neural activities.

Disclosures: D. Yan: None. **D. Jeong:** None. **J.R. Lopez Ruiz:** None. **E. Yoon:** None.

Poster

666. Probing Technologies II

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Program #/Poster #: 666.06

Topic: I.04. Physiological Methods

Support: NIH Grant 2 R44 NS105500-02

Title: Insertion of planar and 3D matrix-style electrode arrays using ultrasonic vibration decreases electrode shank damage while maintaining electrode recording integrity.

Authors: *K. W. GHERES[1], N. N. TIRKO[2], J. K. GREASER[1], A. S. ALSUBHI[1], R. B. BAGWELL[1], M. L. MULVIHILL[1];
[1]Actuated Med. Inc., Bellefonte, PA; [2]Eberly Col. of Sci., The Pennsylvania State Univ., University Park, PA

Abstract: Intracortical electrode arrays provide precise spatial recording of extracellular neural signals with high temporal precision, however, chronic recordings lose recording fidelity over the time due to the host foreign body response. Two approaches to minimize the foreign body response are use of small diameter (<20μm) electrode shanks and “floating” arrays which move with the brain decreasing stress at the electrode-tissue interface. Small diameter carbon fiber

electrode shanks have demonstrated a decreased foreign body response but have a lower absolute buckling force, requiring secondary support during insertion. As vibrated insertion has been shown to decrease required force of insertion for many electrode arrays, we investigated whether high density carbon fiber arrays (University of Michigan MINT) could be inserted with less damage to the probes using the NeuralGlider Inserter. Compared to control insertions, vibrated insertion of MINT arrays decreased peak insertion force in a bench top model and resulted in more active recording channels in chronic recordings. Three dimensional (3D) multi-shank floating arrays such as the NeuroNexus Matrix Array^{TM} provide high density recordings but can cause significant tissue deformation due to their dense shank spacing. Current insertion methods rely on broad shank spacing and high insertion speeds (4.5mm/s) to minimize insertion-based tissue deformation. We developed a new coupling mechanism for 3D arrays which uses vacuum pressure to hold arrays to NeuralGlider while vibrating using slow insertion conditions (0.05mm/s). We tested the ability to completely insert 3D arrays with the new coupling mechanism in a juvenile porcine model. Like other larger mammals, the porcine arachnoid and pial membranes are thicker and requires higher forces for electrode insertion, making complete insertion of multi-shank arrays difficult at low speeds. Using the NeuroNexus Matrix Array (4x4, 200µm shank spacing, 1000µm platform spacing, 2mm shank length) we demonstrated that vibrated insertion with NeuralGlider Inserter (0.75W) can reliably implant multi-shank arrays into porcine cortex without electrode shank damage. Electrophysiology recordings following implantation displayed spontaneous neural activity across channels in subjects within an hour following device implantation confirming device integrity. These results demonstrate that vibrated insertion provided by the NeuralGlider Inserter facilitates damage-free implantation of delicate small diameter carbon fiber arrays and can enable low-speed insertion of silicon multi-shank arrays without damage into larger mammalian model organisms.

Disclosures: **K.W. Gheres:** A. Employment/Salary (full or part-time):; Actuated Medical Inc. **N.N. Tirko:** F. Consulting Fees (e.g., advisory boards); Actuated Medical Inc. **J.K. Greaser:** A. Employment/Salary (full or part-time):; Actuated Medical Inc. **A.S. Alsubhi:** A. Employment/Salary (full or part-time):; Actuated Medical Inc. **R.B. Bagwell:** A. Employment/Salary (full or part-time):; Actuated Medical Inc. **M.L. Mulvihill:** A. Employment/Salary (full or part-time):; Actuated Medical Inc..

Poster

666. Probing Technologies II

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Program #/Poster #: 666.07

Topic: I.04. Physiological Methods

Support: NIH 1RF1NS113303

Title: Flexible Parylene photonic neural probes for in vivo optogenetic manipulation of neurons

Authors: J. REDDY[1], V. JAIN[2], *M. CHAMANZAR[3];
[2]Electrical and Computer Engin., [1]Carnegie Mellon Univ., Pittsburgh, PA; [3]Carnegie Mellon, Pittsburgh, PA

Abstract: New neuroscience tools for light delivery in-vivo, such as implantable neural probes, are necessary to enable precise genetically-targeted optical neural stimulation via optogenetics. Optical waveguide arrays and microLEDs have been integrated in compact optical neural probes composed of rigid substrate materials to deliver light to neural tissue. However, a flexible material platform is preferred for chronic interfacing with the brain, since rigid materials can damage the soft brain tissue due to different factors such as brain micromotions. Parylene photonics offers a flexible, biocompatible integrated photonic material platform composed of a Parylene C core (n = 1.639) and PDMS cladding (n = 1.4, Δn = 0.239). Here, for the first time, we demonstrate light delivery for in-vivo optogenetic stimulation using 10 μm Parylene photonic waveguides on an implantable neural probe. The devices are fabricated using a high-throughput wafer-scale microfabrication process. Individual fully-flexible neural probes are released from the wafer and packaged with optical fibers to deliver light. Implantation of the flexible probes is achieved using a Tungsten shuttle, which is attached to the probe using bio-dissolvable Polyethylene Glycol (PEG). We also demonstrate flexible Parylene photonic neural probes as versatile tools to add optical functionality to existing commercial electrode arrays by laminating the flexible waveguide array to a rigid Si neural probe. Optogenetic stimulation using Parylene photonics is shown by recording the evoked activity in transgenic mice expressing ReaChr opsin. Spike sorting resulted in identifying 23 distinct single units in the recorded dataset, showing both spontaneous and optically-evoked activity. The presented results demonstrate the practical use of Parylene photonics to enable less-invasive neural stimulation.

Disclosures: J. Reddy: None. **V. Jain:** None. **M. Chamanzar:** None.

Poster

666. Probing Technologies II

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Program #/Poster #: 666.08

Topic: I.04. Physiological Methods

Support: R01NS089688
U01NS113279
R01NS062019

Title: Stabilizing L1CAM on Parylene-C surface to enable the dissemination of biomimetic coating technologies

Authors: *D. SHI[1,2], V. DHAWAN[1,2], T. CUI[1,2,3];
[1]Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for Neural Basis of Cognition, Pittsburgh, PA; [3]McGowan Inst. of Regenerative Med., Pittsburgh, PA

Abstract: Foreign body response (FBR) in the brain tissue is one of the major causes of the electrophysiological signal degradation for chronically implanted microelectrodes such as the Utah arrays. Applying a biomimetic coating on the electrode surface has been an effective strategy to attenuate the FBR and to promote neuronal health. Among a wide range of bio-derived molecules, protein-based coatings stand out for their unique bioactivities which enable more precise modulation of cellular responses for neurons and glial cells. For example, Golabchi et al. demonstrated that neuroadhesive glycoprotein L1CAM can enhance chronic electrophysiological performance for up to 16 weeks in rodents. However, most protein-based biomolecules are fragile, susceptible to denaturation, and can lose their bioactivity without sophisticated preservation methods. As a result, most protein-based coating applications are limited to on-site usage because the common commercial storage and shipping conditions can be detrimental to proteins. Here, we evaluated the ability of EDC (1-ethyl-3-(3-) carbodiimide) / NHS (N-Hydroxysuccinimide) cross-linking chemistry to stabilize the L1CAM on the parylene-coated surface. We tested the bioactivity of crosslinked L1CAM over different storage temperatures and time points using *in vitro* neurite growth assay (Woeppel et al. 2021). Borosilicate glass wafers were coated with parylene-C through chemical vapor deposition to simulate the surface chemistry of the Utah arrays. The wafers were diced into smaller pieces and treated with oxygen plasma, followed by immobilization of L1CAM via EDC/NHS crosslinker, and stored until ready for cell culture. After quantifying the neurite growth, we found that the crosslinked L1CAM can maintain its bioactivity for up to 10 weeks when stored at -20°C and for up to 2 weeks when stored at room temperature. This crosslinking method enables the shipping of electrode arrays coated with fragile protein-based biomolecules to global users. The dissemination of such biomimetic coating technologies can greatly benefit the scientific community by enhancing chronic electrophysiological performance. We have also started *in vivo* testing with 4X4 Utah arrays implanted in the rat motor cortex. To compare the effect of the L1CAM coating within the same array, we have developed a 3D printed selective coating device that enables us to selectively coat alternate shanks and leave the uncoated shanks as internal controls. Ongoing chronic free-moving electrophysiological recording, endpoint histology, and explant analysis will reveal more information on how the biomimetic coating interacts with the neighboring tissue.

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Poster

666. Probing Technologies II

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Program #/Poster #: 666.09

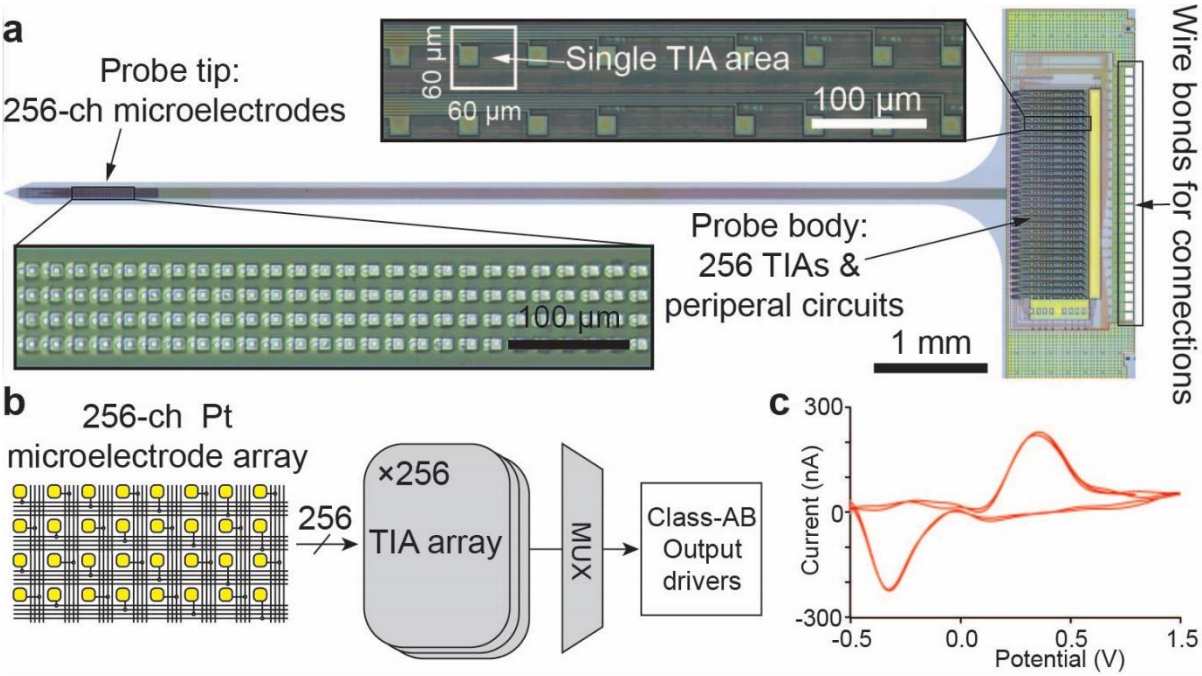
Topic: I.04. Physiological Methods

Support: NSF Grant 2133225
NSF Grant 2143140

Title: High-density neurochemical MEA probe for dopamine mapping

Authors: *B. N. KIM, K. A. WHITE;
Electrical and Computer Engin., Univ. of Central Florida, Orlando, FL

Abstract: Dopamine constitutes about 80% of the catecholamine content in the brain and is involved in cognitive processes and reward-motivated behaviors. As we are only beginning to understand the intricate, sub-regional, spatiotemporal distribution of dopamine innervation in the brain, there is a critical need for a new neurotechnology that can map spatially and temporally distributed neurochemicals. High-density mapping of neurochemical activities with high spatiotemporal resolution is not possible with existing technologies such as CFE electrodes (low spatial resolution) and neurochemical fMRI (low temporal resolution). In this work, we present a new high-density 256-ch neurochemical microelectrode array (MEA) probe that can map neurochemicals with a high spatiotemporal resolution (Fig. a). The probe integrates 256-ch on-chip platinum microelectrodes and on-chip signal-processing units including 256 transimpedance amplifiers (TIAs), multiplexers, and output buffers (Fig. b). Each TIA is designed to enable electrochemical detections of electroactive molecules, such as dopamine, in two distinct modes: amperometry and fast-scan cyclic voltammetry (FSCV). The amperometry mode has the benefit of high temporal resolution (25 µsec) while FSCV can achieve high sensitivity (~10 nM). The probe can perform fully parallelized FSCV from all 256 electrodes using 256 on-chip amplifiers. The probe is first fabricated using a standard 0.35 µm CMOS process into a microchip, and subsequently, on-chip platinum microelectrodes are integrated directly onto the microchip using post-CMOS processing (each platinum electrode is 5 µm in diameter). Finally, the microchip is shaped into a shank using multiple reactive-ion etching (RIE) steps. The probe is tested by measuring ferrocyanide at 300V/s (Fig. c). This novel neurochemical MEA probe can be used to identify the distinct role of heterogeneous distribution of dopamine, both spatially and temporally, in neuromodulations including directing motor control, motivation, reward, and cognitive function.



Disclosures: **B.N. Kim:** None. **K.A. White:** None.

Poster

666. Probing Technologies II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 666.10

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS104344-02

Title: Force Insertion and Acute Electrochemical Measurements of Amorphous Silicon Carbide Microelectrode Arrays into Nonhuman Primate Motor Cortex

Authors: *J. R. ABBOTT[1], N. GERAMIFARD[1], A. G. HERNANDEZ REYNOSO[1], K. P. BLUM[2], Y. WU[1], L. E. MILLER[2], S. F. COGAN[1];
[1]Univ. of Texas at Dallas, Richardson, TX; [2]Northwestern Univ., Chicago, IL

Abstract: Chronically implanted microelectrode arrays in nonhuman primates (NHP) have been used to record neural electrical activity. Recent studies have shown that recording capabilities of some implanted arrays can decrease during chronic studies. Possible causes include tissue encapsulation by the foreign body response exacerbated by large cross-sectional geometries of implanted devices and mismatch of stiffness between the device and brain tissue. Amorphous silicon carbide (a-SiC) probes with cross-sectional dimensions of 120 μm^2 have been shown to chronically record neural activity from rat motor cortex. However, NHP pial tissue is stiffer than rat, which creates challenges for inserting devices with small cross-sectional areas without buckling. In this study, we measured the required insertion forces using single shank a-SiC probes with different lengths and cross-sectional dimensions during an insertion into cortex. These data will inform the required dimensions for pial penetration of future multielectrode arrays for chronic recording in NHP cerebral cortex. Multi-shank devices were also implanted, and electrochemistry was measured in an acute preparation to measure functionality post-surgery. Required insertion forces were measured during insertion using a force transducer attached to a fixed rate motorized drive. These probes had thickness x width x length dimensions (probe ID) of 8 µm x 50 µm x 1 mm (1), 10 µm x 50 µm x 2 mm (2), 10 µm x 20 µm x 2 mm (3), and 10 µm x 20 µm x 1.5 mm (4). Three functional 4-shank, 32-channel a-SiC devices based on probe 4 with 200 μm^2 sputtered iridium oxide electrodes were also implanted. 1 kHz impedance and voltage transient electrical stimulation measurements were recorded acutely.Type 1 probes had 3 successful insertions with minimal or no buckling. Type 2 probes had moderate success, inserting once and then buckling on the next insertion attempt. Type 3 had no successful insertions. Type 4 probes consistently had a slight buckle and then successfully inserted into the cortex. Across all probes that successfully inserted, we measured an insertion force of 0.72 ± 0.26 mN (1 SD) required for a-SiC probes to penetrate the pia. The functional multi-shank devices were more challenging to insert than single shank probes. Despite these challenges, we were able to record electrochemical data across 12 electrode sites. We measured a charge

injection capacity of 0.51 ± 0.05 mC/cm^2 and an average 1 kHz impedance of 1.05 ± 0.12 MΩ over 12 channels. In future chronic studies in NHP, we will use several single shank devices to improve successful insertions.

Disclosures: **J.R. Abbott:** None. **N. Geramifard:** None. **A.G. Hernandez Reynoso:** None. **K.P. Blum:** None. **Y. Wu:** None. **L.E. Miller:** None. **S.F. Cogan:** None.

Poster

666. Probing Technologies II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 666.11

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS110823
United States Department of Veterans Affairs Rehabilitation Research and Development Service grant IK6RX003077

Title: The effect of an antioxidant coating on the acute recording performance of planar silicon intracortical microelectrode arrays

Authors: *A. G. HERNANDEZ-REYNOSO[1,2], O. KREBS[3,4], B. STURGILL[1], G. HOEFERLIN[3,4], J. ZHANG[3,4], G. MITTAL[3,4], T. T. D. THAI[1], M. S. DESAI[1], S. F. COGAN[1], J. J. PANCRAZIO[1], J. R. CAPADONA[3,4];
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Abstract: Intracortical Microelectrode Arrays (MEAs) can record neural activity from the brain and are used to develop brain-machine interfaces and to advance understanding of brain circuitry. However, implantation of these devices initiates a neuroinflammatory cascade, potentially resulting in reactive oxygen species that accumulate around the implant site, resulting in neuronal dysfunction. This process may contribute, at least in part, to the failure at the interface because the ability of MEAs to record is related to the proximity of viable neurons around the implant. Our group has developed a sustained antioxidant coating based on the immobilization of mimetic superoxide dismutase – a protective enzyme that degrades harmful superoxide anions into less reactive elements – to improve the longevity of recording MEAs by attempting to modulate the accumulation of reactive oxygen species around the implant. Here, the goal was to investigate the effect of these coatings to the MEA acute recording performance in-vivo. Animal procedures were approved by the University Texas at Dallas IACUC. Sprague Dawley rats were implanted with coated or uncoated (control) MEAs targeting the primary motor cortex (M1) of the brain. Neurophysiological data were recorded weekly for 10 minutes under anesthesia at a sampling rate of 40 kHz for weeks after implantation and then filtered

between 300-3000 Hz. Individual spike waveforms were extracted using a -4σ threshold and sorted using K-means. The active electrode yield was then calculated as the proportion of total electrode sites that captured at least one single unit. A two-proportion z-test was used to calculate statistical differences between groups with $p<0.05$ considered as significant.Coated and uncoated MEAs showed similar proportion of active electrodes upon implantation (approximately ~43%). Both groups experienced an almost two-fold increase in the number of active electrodes after 1 week (approximately ~77%); however, there was no statistically significant difference between groups. Whereas the uncoated MEA group demonstrated a constant loss of active electrodes, the coated group experienced fluctuations in yield of ~10% thereafter. Despite these fluctuations, eight weeks after implantation, there was a statistically significant difference in the proportion of active electrodes between the two groups ($p < 0.05$).These results suggest that coating MEAs with an antioxidant can significantly improve the reliability of these devices to acutely record neural activity. Future studies should evaluate the effect of these coatings on the chronic performance of MEAs.

Disclosures: **A.G. Hernandez-Reynoso:** None. **O. Krebs:** None. **B. Sturgill:** None. **G. Hoeferlin:** None. **J. Zhang:** None. **G. Mittal:** None. **T.T.D. Thai:** None. **M.S. Desai:** None. **S.F. Cogan:** None. **J.J. Pancrazio:** None. **J.R. Capadona:** None.

Poster

666. Probing Technologies II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 666.12

Topic: I.04. Physiological Methods

Support: NIH Grant DP2NS122605
NIH Grant K01EB027184
Wisconsin Alumni Research Fund
NSF Grant DMR-1720415

Title: Validation of T2 relaxation rates following superparamagnetic iron oxide nanoparticle aggregation for sensing of neural processes

Authors: *I. BOK[1], B. RAUCH[2], A. HAI[1];
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Abstract: Emerging injectable sensors for magnetic resonance imaging (MRI) of brain signaling rely on the aggregation of chemically functionalized superparamagnetic iron oxide nanoparticles (SPIONs) for detecting calcium fluctuations, neurotransmitter dynamics, and other neurobiological phenomena. Computational models of SPION clustering demonstrate optimized MRI T2 response based on SPION size, clustering behavior, and interparticle distance. Experimentally validating these predictions will significantly impact new sensor designs and inform therapeutic applications in the brain, ranging from assessment of neuronal recovery to

detection of stroke, neuroinflammation, and other pathologies. Presented here is a novel nanofabrication scheme for generating highly controllable two-dimensional cluster geometries at the nanometer scale and measurements of corresponding MRI T2 relaxation rates. We use electron-beam lithography on a silicon substrate and deposit an ultra-thin layer of iron before lifting off. 100 μm arrays of SPIONs with interparticle-distance to particle-radius ratios ranging from 4 to 60, alongside quasi-isotropic clusters and randomly oriented chains, were fabricated. For cluster signature analysis, histograms of fast spin-echo multi-slice MRI pulse sequences were analyzed to determine the statistically significant dependability of T2 relaxation on array geometry. We report a predictable dose-dependent decrease in T2 signal with increasing density (N=64 voxels each; one-way analysis of variance, p=6.7e-53) within the slow-motion regime, and similarity in T2 signal between chains and quasi-isotropic clusters (N=30 each; no significance, two-sample student's t-test, p=0.398). Our results validate computationally predicted behaviors from previous studies and demonstrate new geometric dependencies with implications for emergent intersensor properties in different tissue types and brain diffusive scenarios. Future fabrication expansion to three-dimensional assemblages will allow for a scalable on-chip evolution of MRI brain sensors.

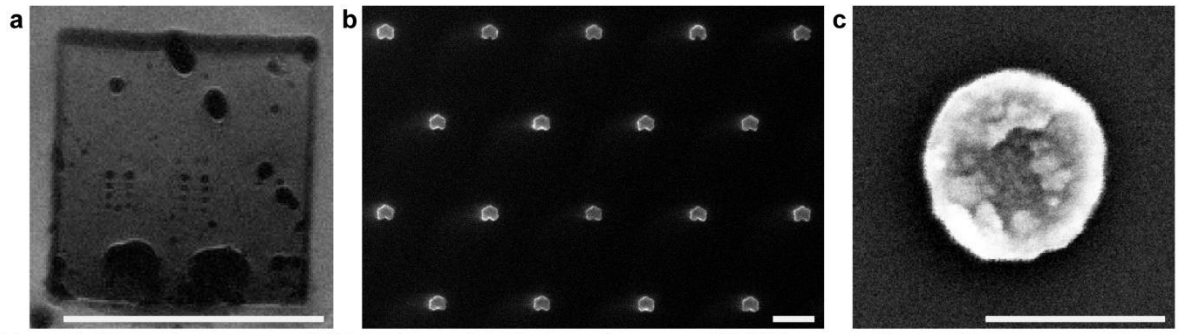

Figure 1: Magnetic resonance (MR) and scanning electron microscopy (SEM) images of iron oxide nanogeometries. a) MR fast spin-echo multi-slice scan of a 10 mm x 10 mm sample with density matrix and alternate geometry squares (above matrix, upper right) visible near the center. Scalebar = 10 mm. b) SEM image of uniform quasi-isotropic clusters consisting of 6 merged nanodots. Scalebar = 1 μm. c) SEM close-up image of a single iron oxide nanodot. Scalebar = 200 nm.

Disclosures: **I. Bok:** None. **B. Rauch:** None. **A. Hai:** None.

Poster

666. Probing Technologies II

Location: SDCC Halls B-H

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Program #/Poster #: 666.13

Topic: I.04. Physiological Methods

Support: NIH RF1NS113278-01 (CQ and XY)
NIH R01AG060731-A1 (AMD and SC)
NIH R21AG074514-01 (AMD)

NIH R01-HL-13769401 (AMD)
EU Marie-Curie No.896245 (YC)

Title: Novel inductively-coupled ear-bars (ICEs) for fMRI signal enhancement in rat entorhinal cortex

Authors: *Y. CHEN[1,2], Z. FERNANDEZ[2,3], D. C. ZHU[2,3], S. E. COUNTS[8,4,5,6,9], A. M. DORRANCE[3,7], X. YU[10], N. SCHEEL[2], W. QIAN[2], M. GIFANI[4], C. QIAN[2];
[1]Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany; [2]Dept. of Radiology, [3]Neurosci. Program, Michigan State Univ., East Lansing, MI; [4]Dept. of Translational Neurosci., Michigan State Univ., Grand Rapids, MI; [5]Cell and Mol. Biol. Program, Michigan State Univ., East Lansing, MI; [6]Dept. of Family Med., Michigan State Univ., Grand Rapids, MI; [7]Dept. of Pharmacol. and Toxicology, Michigan State Univ., East Lansing, MI; [8]Michigan Alzheimer's Dis. Res. Ctr., Ann Arbor, MI; [9]Hauenstein Neurosciences Ctr., Mercy Hlth. St. Mary's Hosp., Grand Rapids, MI; [10]Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: Entorhinal cortex (EC) is a potential target of deep brain stimulation in Alzheimer's disease (AD) rodent models. Studies reported that the lateral EC is the earliest forebrain area affected by AD, as shown by neurofibrillary tangles. However, it remains challenging to study the EC-based fMRI connectivity in rats due to image signal loss and the lower sensitivity of the surface coil ring or array coil for the deep brain areas, in particular, EC. The fMRI image signal loss at EC is due to its proximity to the air-tissue interface in the ears which introduces a high level of magnetic field inhomogeneity. To reduce the signal loss issue, we introduced baby cream (Baby Healing Ointment, Meijer) into the middle ear. To address the sensitivity issue of the coil, we implemented two inductively coupled ear-bars (ICEs) in the 7T Bruker scanner to relay locally detected MR signals to the external coil array with a nearly 2-fold signal-to-noise in EC over the conventional surface array increase. We now can acquire high-quality functional images of the rat brains with restored fMRI signal in the EC (spatial resolution: 0.5 mm × 0.5 mm × 0.5 mm, TR: 1s), along with high-quality high-resolution structural images in dexmedetomidine-anesthetized dementia rats. We evaluated this setup by measuring the fMRI signal over the whole brain first using task fMRI with electrical stimulation on the left forepaw (3 Hz, 4 s, 3 mA stimulation) and then using resting-state fMRI (rs-fMRI). In the task fMRI, we observed a robust evoked BOLD signal along with the ascending projection to the motor cortex from the thalamus. In the rs-fMRI, we observed a strong default-mode network, including prelimbic cortex, cingulate cortex, auditory cortex, posterior parietal cortex, and retrosplenial cortex. Finally, we analyzed the seed-based rs-fMRI connectivity maps based on the left EC and observed strong connectivity in the hippocampus, piriform cortex, septal nuclei, and prefrontal cortex. In summary, our optimized ICE-based procedure enables and facilitates EC-driven brain fMRI studies with a simplified experimental setup and provides the possibility to further study EC in AD rat models.

Disclosures: Y. Chen: None. **Z. Fernandez:** None. **D. C. Zhu:** None. **S. E. Counts:** None. **A. M. Dorrance:** None. **X. Yu:** None. **N. Scheel:** None. **W. Qian:** None. **M. Gifani:** None. **C. Qian:** None.

Poster

666. Probing Technologies II

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

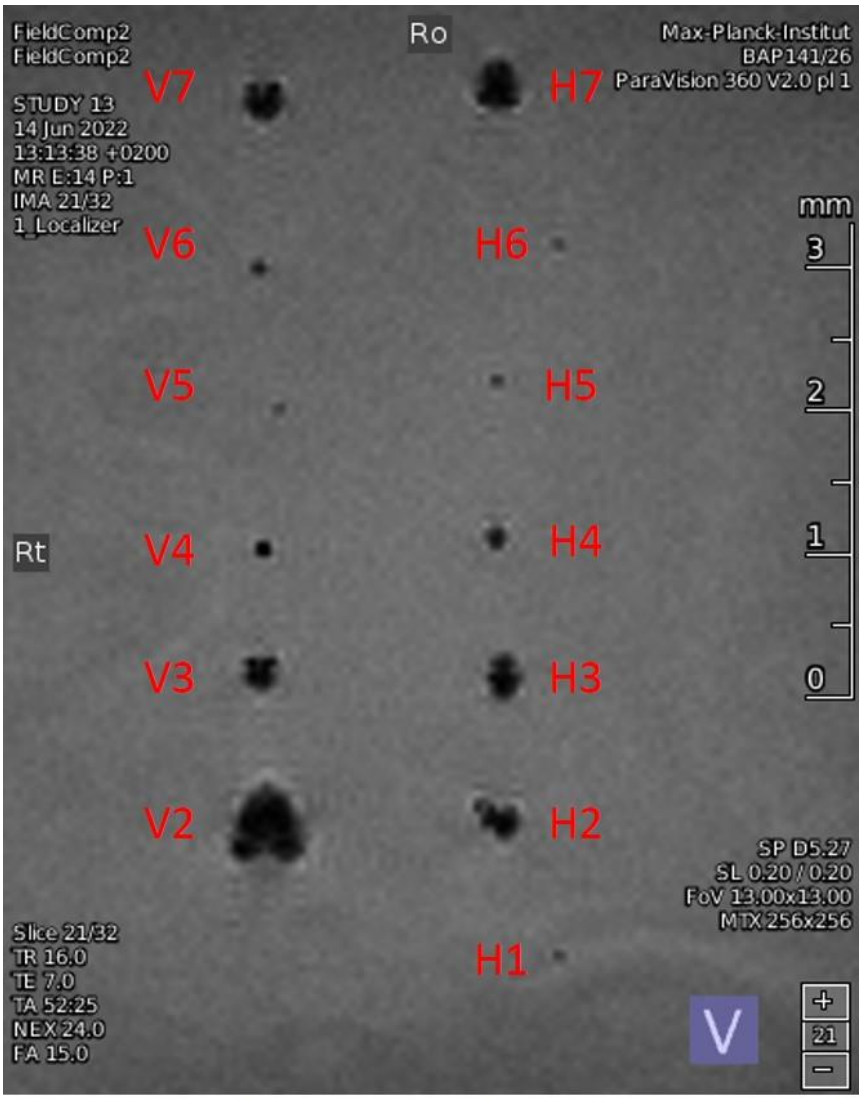
Program #/Poster #: 666.14

Topic: I.04. Physiological Methods

Title: Tissue-susceptibility matched electrodes for simultaneous magnetic resonance imaging

Authors: *A. VON RAVEN, A. OELTERMANN, R. POHMANN, K. SCHEFFLER;
Max Planck Inst. For Biol. Cybernetics, Tübingen, Germany

Abstract: Introduction: Deep Brain Stimulation (DBS) is a validated technique to treat many neurological diseases. Post-surgical imaging is currently done with magnetic resonance imaging (MRI), which, due to susceptibility artifacts around electrodes, can result in their imperfect localization. Most DBS electrodes are made of a Pt-Ir (90%/10%) alloy, which exhibits good conductivity, biocompatibility and resistance to corrosion, but causes large artifacts due to susceptibility differences to tissue. Developing new electrodes to better match tissue susceptibility using carbon monofilament electrodes is difficult due to their electrical and mechanical properties. By combining materials that alone do not have the mechanical, electrical or biocompatible properties, we aim to design electrodes with improved MRI characteristics. **Methods**: Copper is a flexible, non-biocompatible metal with great conductivity and susceptibility properties. Combined with a Polyurethane insulation, tin coating at the tip and an additional fiber for stabilization it could work as low-susceptibility (multi-channel) electrode. The coating was done using a galvanization method. To test the susceptibility properties, gradient echo images of different electrode materials were acquired in a 14 T MR scanner. **Results**: It is clearly visible that the copper wires H1, H6 and V5 show the lowest susceptibility artifact, independent of whether the copper wire is insulated and/or coated. The silver plated copper alloy shows a slightly higher susceptibility artifact than the pure copper wires. Pt/Ir, NeuroNexus and carbon fiber perform worst. **Discussion**: The use of insulated copper wire in combination with biocompatible coating can be considered as alternative to conventional electrodes with bigger susceptibility artifacts. This gets even more important, since there is clearly a trend to use higher field strengths. In addition to DBS, those electrodes can also be used for animal experiments where the combination of electrophysiology and fMRI experiments can offer valuable insights into brain function.



TR: 16 ms; TE: 7 ms; flip angle: 15 degree. The following materials were scanned: H1: single isolated copper wire, Ø 20µm, with tin coating; H2 – H5: single/multiple SiC and Al2O3 fiber for stiffness; H6: single isolated copper wire 20µmØ; H7: commercial NeuroNexus electrode on a silicon base with 16 channels; V2: single Pt-Ir (90%/10%) wire 25µm Ø; V3: single carbon fiber, Ø 35µm; V4: single silver plated copper alloy, Ø 20µm; V5: double isolated copper wire, Ø30µm, V6: copper wire, Ø 40 µm; V7: carbon fiber with isolation of borosilicate glass, Ø40µm.

Disclosures: **A. von Raven:** None. **A. Oeltermann:** None. **R. Pohmann:** None. **K. Scheffler:** None.

Poster

666. Probing Technologies II

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Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 666.15

Topic: I.04. Physiological Methods

Support: NIH Grant UF1NS107676

Title: Chronic oxygen measurements around soft and ultrasoft neural interfaces

Authors: *A. SRIDHARAN, Y. SUGAMURA, L. DE MESQUITA TEIXEIRA, V. KODIBAGKAR, J. MUTHUSWAMY;
Sch. of Biol. & Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

Abstract: Local inflammatory processes and chronic mechanical stresses are seen around chronic neural interfaces, which appear to be mitigated by softer materials. However, the role of oxygenation due to the above processes and the effect on neuronal function is unknown at this point. This study compares local oxygenation in brain tissue around soft (elastic modulus, E of 100-300 kPa) and ultra-soft (elastic modulus, E of 1-5 kPa) neural interfaces under chronic conditions. We use a novel MR-based oximetry technique (PISTOL-Proton Imaging of Siloxane Tissue Oxygen Levels) to measure pO2 levels over seven weeks of implantation. Carbon fiber probes (~50 × 20 micron cross-section) were coated with either soft (E~100-300 kPa) or brain-like ultrasoft (E~1-5 kPa) polydimethylsiloxane (PDMS) matrix incorporated with carbon nanotubes and oxygen-sensitive tetradecamethylhexasiloxane (L6). The overall diameter of these neural interfaces ranged from 170-220 microns and was implanted in six mice with two soft and two ultrasoft probes in either hemisphere of each animal. Seven ultrasoft probes and three soft probes yielded oxygen measurements for subcortical regions (up to 2 mm below the cortex). Mean baseline oxygenation levels ranged from 60-100 Torr for ultrasoft probes (n=7) at 0 and 7 weeks post-implantation. For 2 of the three soft probes, mean oxygenation levels ranged from 10-50 Torr, and one probe had 70-100 Torr. Oxygen measurements using commercial sensors with ultrasoft coatings (n=8 measurements from 2 mice) in acute experiments validate baseline oxygenation levels (40-90 Torr) in subcortical regions (1.5-3 mm depth) in vivo. The above results suggest that neural interfaces made of materials that are not mechanically matched to the surrounding brain tissue can induce lower oxygenation levels in chronic experiments, which could potentially lead to neuronal dysfunction and exacerbation of inflammatory processes through reactive oxygen species. Further, quantitative assessments of local oxygenation levels can lead to strategies for mitigating tissue damage around implantable neural interfaces for neuromodulation.

Disclosures: A. Sridharan: None. **Y. Sugamura:** None. **L. de Mesquita Teixeira:** None. **V. Kodibagkar:** None. **J. Muthuswamy:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.01

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH T32 EB006359-06
NSF 1633516

Title: Linear Dimensionality Reduction for Neuronal Network Analysis Under Isoflurane Sedation in Mice

Authors: *D. CARBONERO[1,2,3], J. NOUEIHED[1,2,3], J. A. WHITE[1,2,3];
[1]Biomed. Engin., [2]Neurophotonics Ctr., [3]Ctr. for Systems Neurosci., Boston Univ., Boston, MA

Abstract: Anesthetics have become vital to modern medicine, allowing for painless performance of otherwise intolerable medical procedures. Mechanisms and effects of volatile anesthetics are well understood at the level of ion channels and the level of activity changes across entire brain regions. However, only a few studies have focused on the mechanisms of anesthesia at the mesoscale level and focused on neuronal networks and sub-networks. Using calcium imaging in layer 2/3 of S1, we recorded network activity from hundreds of cells and quantified cell output at varying concentrations of anesthesia (0%, 0.7%, and 1.4% isoflurane by volume).
Our initial analyses, using averaged single neuronal responses measured with Ca^{+2} imaging, indicate that excitatory and inhibitory activity is balanced in S1 during steady state concentrations of anesthesia. Further, pairwise correlations of neuronal activity increase significantly under increasing anesthesia. Nevertheless, given the drastic behavioral changes associated with the onset of anesthesia, changes in network correlations in layer 2/3 S1 were relatively modest.
To further explore changes induced by anesthesia, we adapted a series of linear dimensionality reduction (DR) methods to represent activity at different levels of anesthesia at a much lower dimensionality, while maintaining uniqueness found in each neuronal response. Leveraging the mathematical relationships of these models, we represented network activity as a series of sub-networks or smaller patterns of activity, with each neuron contributing a weight to that pattern of activity. Our models indicate that anesthesia decreases the magnitude of activity, with activity also becoming more uniform or correlated. The shift seen in the fit model under different concentrations of anesthesia suggest a shift from a complex structure of activity, with several active sub-networks competing to drive activity, to one in which a single structure of activity dominates. While our cursory analyses are in line with previous work on anesthesia (maintained balanced, increase in correlation), our fit DR models can convey further information about the complex network activity using a low dimensional sub-network representation.

Disclosures: D. Carbonero: None. **J. Noueihed:** None. **J.A. White:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.02

Topic: I.06. Computation, Modeling, and Simulation

Support: NRF-2022R1A2C3008991
NRF-2019M3E5D2A01058328
NRF-2021M3E5D2A01019544

Title: A small-world network model predicts the species-specific emergence of cortical long-range circuits

Authors: *S. BAEK[1], Y. PARK[1], S.-B. PAIK[1,2,3];
[1]Dept. of Bio and Brain Engin., [2]Program of Brain and Cognitive Engin., [3]Dept. of Brain and Cognitive Sci., KAIST, Daejeon, Korea, Republic of

Abstract: Long-range horizontal connections (LRCs) refer to extraordinarily long wiring (up to 2-3mm), distinguished from local connections (up to 1mm), that are observed in the primary visual cortex (V1) of various species. Observations across species with (rat; Rumberger 2001) or without (mouse; Seeman 2018) LRCs suggest that they have a distinct function for visual processing under species-specific conditions of V1. However, the species-specific existence of LRCs and their possible roles are still not fully understood. Here, using a computational model of simplified cortical circuits, we suggest that LRCs with local connections can organize a "small-world network" (Watts and Strogatz 1998) in V1 and that this can provide an explanation of the emergence of LRCs across species with different cortical sizes. From the definition of the "small-world" network that minimizes the average distance between distant nodes while maximizing the degree of clustering between adjacent nodes with a limited number of connections, we hypothesized that the "small-world" structure in V1 enables the integration of visual information over a wide spectrum range, with this structure possibly organized spontaneously from different circuitry profiles depending on the network size. Specifically, in a large V1, LRCs with local connections can construct a "small world" that integrates a broad range of visual information, while they may not be advantageous in a small V1, where only local connections suffice. To validate this hypothesis, we implemented a convergent network model as a model of retino-cortical projections and trained the network to classify image sets while varying the ratios of the LRCs and network sizes. We found that the addition of LRCs increases the classification accuracy in large networks and, notably, that the performance of the network was maximized with a certain proportion of LRCs. We also confirmed that the performance and small-worldness (SW) were strongly correlated in most conditions. This result suggests that the SW can represent the optimal connectivity of the network to encode visual information. Furthermore, LRCs increase the performance only when the network size exceeds a certain threshold, as predicted by the SW model. Overall, our results suggest that a combination of distinct types of cortical connectivity induces a mathematically optimal profile of circuitry, thus providing an understanding of the species-specific existence of LRCs in mammalian species.

Disclosures: S. Baek: None. **Y. Park:** None. **S. Paik:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.03

Topic: I.06. Computation, Modeling, and Simulation

Support: PICT 2016 2195

Title: A general method for studying neural dynamics and connectivity in networks of binary threshold neurons

Authors: C. J. MININNI[1], *B. S. ZANUTTO[2];
[1]Inst. de BIología y Medicina Exptl., Buenos Aires, Argentina; [2]Univ. Buenos Aires-CONICET, CABA, Argentina

Abstract: Neural network models are invaluable tools that neuroscientists use to understand how connectivity instantiates dynamics, and how dynamics instantiates computation. Neural networks are usually hand-crafted from experimental data alone, or in combination with algorithms that fit free parameters through optimization of a function objective, such as minimizing error at execution of a relevant behavioral or computational task. Although neural dynamics can be readily found from neural connectivity by simulating the network, the opposite — finding the connectivity that instantiates a desired dynamic — is not a trivial task. Here we present a general method for finding the connectivity that instantiates a given dynamic, in networks of binary threshold neurons. The method, termed "generalized Firing to Parameter" (gFTP), finds the synaptic weight matrix of a recurrent neural network as the solution of a linear system of equations constructed from the target dynamics, stated as the pairwise transitions between population states, and a set of membrane potentials appropriately chosen to assure consistency. We describe the method, and show its utility by constructing and analyzing the connectivity of networks that follow diverse dynamics, like discrete attractors dynamics, as observed in the frontal cortex, or continuous attractor dynamics, as found in the entorhinal cortex.

Disclosures: C.J. Mininni: None. **B.S. Zanutto:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.04

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF PIRE award #1743475
NIH BRAIN Initiative grant R01 NS118606

Title: A Synthetic Nervous System with Coupled Oscillators Controls Peristaltic Locomotion

Authors: *S. RIDDLE[1], W. NOURSE[2], Z. YU[3], P. J. THOMAS[3], R. D. QUINN[1];
[1]Dept. of Mechanical and Aerospace Engin., [2]Dept. of Electrical, Computer, and Systems Engin., [3]Dept. of Mathematics, Applied Mathematics, and Statistics, Case Western Reserve Univ., Cleveland, OH

Abstract: This work showcases the development and analysis of a computational neuroscience model, known as a Synthetic Nervous System (SNS), for the control of a simulated worm robot. An SNS consists of leaky integrator neuron models connected via conductance-based synapses. Using an SNS controller allows for adaptability of the system behavior with minimal changes to the control network. The worm robot kinematics are inspired by earthworm peristalsis which relies on the hydrostatic properties of the worm's body to produce soft-bodied locomotion. In this work the hydrostatic worm body is approximated in two dimensions as a chain of rhombus shaped segments. Each segment has rigid side lengths, joints at the vertices, and a linear actuator to control the segment geometry. The SNS controller utilizes half-center oscillator central pattern generators (CPGs) composed of two mutually inhibited leaky-integrator neurons. The CPG neurons possess voltage gated sodium ion channels that allow additional temporal dynamics to enable pattern generation. The CPG subnetworks are coupled via interneurons and sensory feedback to coordinate segment contractions and produce a peristaltic waveform that propagates down the body of the robot. The neural and synaptic models were tuned via the Functional Subnetwork Approach (FSA) [1]. FSA is a direct analytical tuning method by which the neural and synaptic parameters may be designed to enable a particular subnetwork behavior. This was done using approximated, but realistic, parameter values as found in neuroscience literature. A direct perturbation Floquet multiplier analysis was performed by finding an approximation of the Monodromy matrix for the system to analyze the limit cycle of the peristaltic waveform produced by the network. Since the nontrivial eigenvalues (Floquet multipliers) of the Monodromy matrix were less than one in magnitude, the limit cycle was determined to be linearly stable.
[1] - Szczecinski, N.S., Hunt, A.J., Quinn, R.D.: *A functional subnetwork approach to designing synthetic nervous systems that control legged robot locomotion*. Frontiers in Neurorobotics 11 (2017)

Disclosures: S. Riddle: None. **W. Nourse:** None. **Z. Yu:** None. **P.J. Thomas:** None. **R.D. Quinn:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.05

Topic: I.06. Computation, Modeling, and Simulation

Support: WPI Research Startup

Title: Short-term synaptic plasticity and threshold adaptation cooperate synergistically to yield robust deviance detection in a spiking network model of dissociated culture

Authors: *F. B. KERN, Z. C. CHAO;
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Abstract: Deviance detection is a phenomenon in sensory processing where novel stimuli that violate prior expectations evoke a stronger response than stimuli that are equally infrequent, but not surprising. Previous computational work [1] showed that deviance detection can be produced by spiking network models with a layered feedforward structure and two short-term plasticity mechanisms, namely synaptic depression (STD) and threshold adaptation (TA). While [1] focused on structure as a key feature, we were curious about the contribution of and interaction between STD and TA, hypothesizing that they may work synergistically. We also wondered how the time scales of these mechanisms relate to the range of inter-stimulus intervals (ISI) that allow the network to produce deviant responses.
To investigate these questions, we built a network model of 800 excitatory and 200 inhibitory spiking neurons with STD (τ; = 150 ms) and TA (τ; = 1 s). Following a recent finding of deviance detection in dissociated culture [2], we assigned spatial locations to the neurons and connected them stochastically based on inter-somatic distance, yielding a sparse, non-layered structure reminiscent of cultured networks. We then defined stimulus locations and presented the networks with oddball and random control sequences at ISI from tens of milliseconds to seconds. We find that both STD and TA are individually capable of producing deviance detection in a narrow range of ISI. However, combining the two mechanisms yields networks that robustly respond to deviant stimuli across a wide range of ISI, far exceeding simple addition of the individual effects.
Our results suggest that neural circuits that harness a diverse set of transient plasticity mechanisms covering different time scales may be capable of encoding the temporal structure of their inputs without the need for complex network architecture.
We conclude by drawing parallels to the predictive coding framework with its prediction and error components. We suggest that, while errors may generally be encoded as neural activity, priors and predictions may instead hide in the dynamic state of a circuit, revealing themselves only when probed with appropriate stimulation.
1. Mill R, Coath M, Wennekers T, Denham SL. A Neurocomputational Model of Stimulus-Specific Adaptation to Oddball and Markov Sequences. PLoS Comput Biol. 2011;7. doi:10.1371/journal.pcbi.1002117
2. Tomoyuki Kubota, Kazuhiro Sakurayama, Tomoyo Isoguchi Shiramatsu, Hirokazu Takahashi. Deviance Detection Property in Dissociated Cultures of Neurons. 電気学会論文誌C(電子•情報•システム部門誌). 2021;141: 661-667. doi:10.1541/ieejeiss.141.661

Disclosures: **F.B. Kern:** None. **Z.C. Chao:** None.

Poster

667. Network Computation III

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Program #/Poster #: 667.06

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH grant R01 NS119395

Title: How to infer large-scale anatomical connectivity from LFP-level functional connectivity: a simulation study

Authors: *J. BLOCH, P. ZHANG, D. LEWIS, E. SHEA-BROWN, A. YAZDAN-SHAHMORAD;
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Abstract: Local field potentials (LFPs), or neural voltage fluctuations in extracellular space, are often recorded and studied due to their unique advantages such as stability over time and relative ease of recording compared to neural spikes. One common analysis from LFP signals is the calculation of functional connectivity, or the level of statistical dependence, between recording sites. However, despite the prevalence of LFP functional connectivity metrics, there is minimal understanding of their origin or how they should be interpreted. Here, we develop a simulation of neural activity using the VERTEX platform (Tomsett 2015, Brain Struct. And Funct.) which bridges the gap between neural spikes and LFPs to understand the relationship between neuron-level anatomical connectivity and LFP-level functional connectivity.

The simulation is large-scale and biophysically plausible, having hundreds of thousands of virtual neurons with spiking and connectivity statistics taken from anatomical studies (Binzegger 2004, J. Neuro), and generating LFPs through validated conduction equations. While other simulation studies of LFP functional connectivity exist, they are largely limited by 1) biologically inaccurate models of neural activity and connectivity, 2) biophysically poor models of neuronal LFP generation, 3) a relatively small number of neurons or small spatial scale of the simulation, 4) overly simplistic neural ensemble activity. Our approach addresses each of these, so that our findings can be maximally relevant for large-scale in vivo studies.

Using this platform, we simulate neural activity and the corresponding LFP at a set of virtual electrodes. We quantify LFP-level functional connectivity for a set of directed and undirected metrics, and pairwise and conditional metrics, in multiple standard frequency bands of neuronal oscillations. We compare these values to anatomical level connectivity between recording electrodes in a few different use cases, such as between various cortical layers, within specific cortical layers, for excitatory or inhibitory neurons only or for all neurons, and for different degrees of anatomical connectivity. This analysis allows us to evaluate the advantages and drawbacks of various LFP functional connectivity metrics in different use cases.

Using computational tools uniquely suited to answering this question, with state-of-the-art biophysical accuracy, we uncover the relationship between anatomical connectivity and LFP-level functional connectivity. Our findings can be used to interpret in vivo experimental results and pave the way for more advanced LFP-based interrogation of brain circuits.

Disclosures: J. Bloch: None. **P. Zhang:** None. **D. Lewis:** None. **E. Shea-Brown:** None. **A. Yazdan-Shahmorad:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.07

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant IIS-2023985
NSF Grant 1735095

Title: Synchronous high-amplitude co-fluctuations in functional brain networks during movie-watching

Authors: *J. TANNER[1], J. FASKOWITZ[2], L. BYRGE[5], D. P. KENNEDY[3], O. SPORNS[3], R. BETZEL[4];
[1]Cognitive Sci., [3]Psychological and Brain Sci., [4]Dept. of Psychological and Brain Sci., [2]Indiana Univ., Bloomington, IN; [5]Univ. of North Florida, Jacksonville, FL

Abstract: Recent studies have shown that functional connectivity can be decomposed into its exact framewise contributions, revealing short-lived, infrequent, and high-amplitude time points referred to as "events" (Esfahlani et al, 2020). However, the origin of these events remains unclear. Here, we leveraged inter-subject synchrony during naturalistic movie-watching to address this gap in the literature. Using fMRI data from eight separate movies and two independently collected, processed, and parcellated datasets (n = 129, n= 25 respectively), we found that events synchronize across subjects watching the same movie, and these synchronous events often occur at the end of movie scenes. These events can be further categorized into three types: events that synchronize at the end of movie scenes (boundary events), events that synchronize during the movie (movie events), and events that do not synchronize (asynchronous events). We find that boundary events are distinct from other event types in their co-fluctuation pattern, amplitude and temporal structure (Two-sample *t*-test; Bonferroni corrected, $p_{adj} = 4.76 \times 10^{-5}$), and that they also carry the most subject specific information (Two-sample *t*-test; all p-values $p < 10^{-15}$, unless otherwise indicated). These boundary events are characterized by opposed co-fluctuation between control, salience and visual systems. Additionally, we find that the structure of time-averaged functional connectivity is determined by events that synchronize during movies (i.e. movie events; Two-sample *t*-test $p < 10^{-15}$), and that the co-fluctuation patterns of these events share a time-locked structure (i.e. the similarity of co-fluctuation patterns for movie events depends upon the moment in the movie when they occur; Two-sample *t*-test $p < 10^{-15}$). Finally, we find that this last result is the tail of a more general positive relationship between the degree of event synchronization and the similarity of the whole brain network at that moment in time ($r = 0.47$, $p = 8.81 \times 10^{-6}$). These results suggest a number of testable hypotheses as to the origins of high-amplitude co-fluctuations, or "events". For example, perhaps boundary events are related to discretization of experience (Kurby & Zacks, 2008). Finally, these results contribute to an ongoing dialogue on the nature of events by suggesting—contrary to recent hypotheses that events are simply sampling variability around a fixed correlation structure (Novelli & Razi, 2022; Ladwig et al, 2022)— that events have meaningful, stimulus driven, and correlated structure that should not be found if events arise only due to stochastic fluctuations.

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Poster

667. Network Computation III

Location: SDCC Halls B-H

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Program #/Poster #: 667.08

Topic: I.06. Computation, Modeling, and Simulation

Support: RO1-NS062184
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Title: Imaging molecular functions in the brain with MR contrast agents

Authors: E. L. BEARER[1,2], T. W. USELMAN[1], C. S. MEDINA[1], H. B. GRAY[2], *R. E. JACOBS[3];
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Abstract: Manganese-enhanced magnetic resonance imaging (MEMRI) for T_1 contrast and intravascular ultrasmall super-paramagnetic particles (USPIO) for T_2 hold exceptional promise for preclinical studies of brain-wide physiology. Physiological contrast agents report on how genetic or pharmacologic perturbation of specific molecules affect functions, such as axonal transport, neural activity or cerebral blood flow (CBF). Longitudinal MEMRI offers a powerful platform to witness dynamic effects of genetic manipulations in mice. Mn(II) is powerful for two main reasons: 1) Strong effect at low doses; 2) Biological interactions for neural activity and projection mapping via its entry into electrically active neurons in the living brain and transport within neurons. High-spin Mn(II) reduces relaxation time of nearby water protons: at Mn(II) concentrations typically encountered in MEMRI, robust hyperintensity is obtained without adverse effects. When delivered by stereotactic injection Mn(II) enters active neurons at the injection site and then travels inside axons for long distances, tracing neuronal projection anatomy. When delivered systemically, Mn(II) enters active neurons throughout the brain via voltage-sensitive calcium channels and clears slowly. Thus behavior can be monitored during Mn(II) uptake and hyper-intense signals due to Mn(II) accumulation captured by MRI retrospectively, allowing pairing of behavior in awake behaving mice with brain-wide neural activity maps for the first time. MEMRI has reported effects of classical gene knockouts, chemogenetic activation or inhibition, cocaine addiction and acute threat on brain-wide neural activity. Because of its independence from vascular dynamics, MEMRI may be the only method to witness effects of drugs, like cocaine, that affect both vascular and neural dynamics. We

reported axonal transport dynamics in 8 different genetically altered mice with MEMRI producing clinically useful results for Alzheimer's disease, anxiety disorders and cocaine use. USPIO are powerful for similar reasons, giving a strong effect size for measurement of CBF and useful for measuring effects of drugs or genetics throughout the brain on vasculature and perfusion dynamics. USPIO have been used to reveal brain-wide effects of cocaine and of dopamine on CBF. Automated computational processing allows data-driven, unbiased brain-wide analysis of image stacks from multiple individuals, yielding statistical significance. MEMRI and USPIO thus generate high-resolution 4D images of living brains for physiological measurements and maps of molecular effects, building a framework for multimodal whole brain comparisons.

Disclosures: E.L. Bearer: None. **T.W. Uselman:** None. **C.S. Medina:** None. **H.B. Gray:** None. **R.E. Jacobs:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.09

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant 1835278

Title: Neural Computation in Cortical Cultures

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Abstract: Confined neural networks in vitro can be used to classify spatiotemporal information. Other studies showed that cortical microcircuits are intrinsically responsive to spatiotemporal patterns. This led us to design reservoir computing in cortical cultures that could map a high dimensional network state to its desired output. The neural network is divided into three groups of neurons, 1) Optically stimulated input, 2) Reservoir composed of synaptically connected recurrent network, 3) Output neurons whose activity is detected optically. This experimental setup was designed by confining a cortical network in a polydimethylsiloxane (PDMS) well, and by co-transfecting the neurons with channel-rhodopsin 2 (ChR2) and jRgeco1a. Targeted optical stimulation of input group of neurons was achieved using Polygon 400G which is a patterned stimulator with spatial resolution of approximately 15 µm. In line with other studies, our data showed that neurons in dissociated cortical cultures are very susceptible to synchronized bursts, particularly in the confined network due to synaptic scaling. As these bursts represent chaotic activation and do not carry information about the input, our primary goal was to design an input stimulation protocol to suppress bursts. Our results showed significant reduction of bursts in confined networks when distributed inputs were optically stimulated and each input pattern was

repeated at least once a second. We found that increases in Ca^{2+} baseline level of all neurons was correlated with successful burst suppression. In the absence of population bursts, we expected that high dimensional recurrent network in the reservoir could process spatiotemporal information from the distributed input patterns and store the information in the output readout neurons in real time. From our results, we found strong correlation between outputs and input patterns that indicates processing of spatial information. Next, we plan to evaluate temporal preservation of data by altering the temporal sequence of input patterns. Thus, our in vitro model shows a framework to study reservoir computing with living neural network. One key advantage of this framework is that it will enable us to run learning applications with small sets of training data and computational resources. This model can further be used to study neurocognitive side effects drugs by examining their effects on spatiotemporal processing in living neural networks. Our nonpharmacological method of controlling network wide bursts also has potential applications for the treatment of seizures in epilepsy and tremors in Parkinson's disease.

Disclosures: Z. Ibne Ferdous: None. **Y. Berdichevsky:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.10

Topic: I.06. Computation, Modeling, and Simulation

Support: Labatt Family Innovation Fund in Brain Health

Title: Modelling rTMS-induced metaplasticity dynamics in oscillatory corticothalamic circuits

Authors: *K. KADAK[1], J. D. GRIFFITHS[2];
[1]Med. Sci., [2]Psychiatry, Univ. of Toronto, Toronto, ON, Canada

Abstract: Dysfunction in rhythmic brain circuits, which coordinate a host of cognitive processes, is often observed in many neuropsychiatric diseases, including treatment-resistant depression. Repetitive Transcranial Magnetic Stimulation (rTMS) is an effective treatment for depression, in which a series of patterned magnetic pulses are non-invasively administered to influence brain activity and induce synaptic plasticity. When successful, rTMS results in lasting therapeutic changes in neural circuitry. The salient parameters that comprise rTMS protocols, such as pulse frequency, amplitude, etc., interact to determine its physiological and clinical effects. However, the precise physiological mechanisms through which rTMS-induced effects occur remain poorly understood.
In this work, we present a computational investigation of the neuroplastic effects of intermittent theta-burst stimulation (iTBS), an rTMS protocol class, on oscillatory corticothalamic circuits. The corticothalamic neural field model of Robinson & colleagues, augmented with the plasticity equations introduced and studied by Fung, Wilson, Shouval, & others, provides a compelling biophysical framework for examining how rTMS affects putative physiological mechanisms in

the brain at the meso-macro spatial scale. This model uses a phenomenological description of rTMS-induced synaptic weight changes (long-term potentiation; LTP) based on calcium-dependent plasticity (CaDP) dynamics with a metaplastic sliding threshold scheme. Model simulations using the NFTSim library were run with iTBS parameter ranges of 1-8 pulses-per-burst (PPB) at a 1-10 inter-burst frequency (IBF), applied to excitatory cortical neurons within a four-population corticothalamic network. Pre vs. Post stimulation changes in synaptic weights, as well as simulated resting-state EEG activity, were studied.
We observed that increasing iTBS parameters (PPB & IBF) in the model resulted in monotonic but nonlinear increases in LTP rates. Interestingly, for standard iTBS (3 PPB, 5 Hz IBF), alpha-band (8-12 Hz) oscillations - a signature component of resting-state EEG activity - were significantly suppressed following rTMS (paired t-test; $t = 18.9$, $p < .001$). This is consistent with reports from clinical studies where alpha suppression has been observed following rTMS, with the extent of this suppression being correlated with depression symptom improvement. Our aim is that the computational framework developed here will provide utility for both researchers and clinicians to model, understand, and improve stimulation protocols based on mathematically-characterized rTMS-induced plasticity effects.

Disclosures: K. Kadak: None. **J.D. Griffiths:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

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Program #/Poster #: 667.11

Topic: I.06. Computation, Modeling, and Simulation

Support: CAMH Discovery Fund
Krembil Foundation
UofT EMHSeed Program

Title: Studying brain activity in sleep using mobile EEG, power spectral analysis, and mathematical models of corticothalamic networks

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Abstract: Neurophysiological modelling is a valuable tool for studying the mechanistic underpinnings of brain activity measurements. In this work we use the neural field model developed by Robinson et al. to study corticothalamically-driven activity patterns in electroencephalography (EEG) data. The model describes an analytic power spectrum, derived from its field equations, the shape of which is largely defined by a series of gain (G) parameters, representing connection strengths between cortical and thalamic neural populations. The model

also allows a dimensionality-reduction step, transforming the parameters into an interpretable 3D parameter space with axes x, y, and z representing cortical, corticothalamic, and thalamic feedback levels, respectively. Our objective here is to explore the performance, characteristics, and usability of this modelling approach in the analysis of sleep recordings from modern mobile EEG devices. We used this model to study the physiology of sleep state transitions, using overnight sleep EEG recordings from three sources: 1) The EDF-X Dataset, 197 recordings sampled at 100 Hz. 2) Dreem Open Dataset, 80 recordings, sampled at 250 Hz. 3) A set of 20 recordings collected in-house using the Muse S mobile EEG device, sampled at 256 Hz. Datasets 1 and 2 were professionally scored and dataset 3 was labelled using an automated sleep scoring algorithm. The Robinson model was fitted to these spectra using a Markov Chain Monte Carlo method implemented in the MATLAB toolbox Braintrak, yielding a time series of parameter estimates for corticothalamic connectivity and other physiological parameters throughout the night and across sleep stages. We observe that the fitted and empirical power spectra include similar peaks, characteristic of the corresponding canonical sleep stages. We further replicated previous work using this model and other physiological studies, demonstrating that in slow wave sleep, the parameters corresponding to cortical excitability are heightened, and those related to thalamic relay nuclei excitability are reduced. During this period, y values are the most negative, signifying strong inhibitory thalamocortical activity. In contrast with previous literature, in our study, most y values during wakefulness are negative. We have demonstrated here the feasibility of studying physiological properties of the corticothalamic system from mobile EEG systems using mathematical models of neural activity generation. The ease-of-use and low cost of these systems also opens the door for clinical recordings from large control and clinical populations across a variety of neurological and neuropsychiatric disorders.

Disclosures: **T. Morshedzadeh:** None. **R. Hu:** None. **J.D. Griffiths:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

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Program #/Poster #: 667.12

Topic: I.06. Computation, Modeling, and Simulation

Support: Krembil Foundation
CAMH Discovery Fund
Labatt Family Network

Title: Tms-evoked responses are driven by recurrent large-scale network dynamics

Authors: *D. MOMI[1], Z. WANG[1], J. D. GRIFFITHS[2];
[1]Krembil Ctr. for Neuroinformatics - CAMH, Toronto, ON, Canada; [2]Psychiatry, Univ. of Toronto, Toronto, ON, Canada

Abstract: The brain is a complex and intricately interconnected physical system whose laws of motion and principles of organization have proven challenging to study with currently available measurement techniques. In such circumstances, application of systematic perturbations and measurement of their effects is a central tool in the scientific armoury. In humans, this non-invasive perturbation-based modus operandi is applied by combining transcranial magnetic stimulation (TMS) and electroencephalography (EEG). Spatiotemporally complex and long-lasting TMS-EEG responses are believed to result from recurrent, re-entrant activity that propagates broadly across multiple cortical and subcortical regions, dispersing from and later re-converging on, the primary stimulation site. In particular, the middle and late components of trial-averaged TMS-EEG evoked potential (TEP) waveforms are often taken as key markers of recurrent large-scale network activity and excitability. However, if we loosely understand the TEP of a TMS-stimulated region as the impulse response function of a noisy underdamped harmonic oscillator, then multiple later activity components (waveform peaks) should be expected even for an isolated network node in the complete absence of recurrent inputs. Thus emerges a critically important question for basic and clinical research on human brain dynamics: what parts of the TEP are due to purely local dynamics, what parts are due to reverberant, re-entrant network activity, and how can we distinguish between the two? To disentangle this, we combined source-localized TMS-EEG analyses and whole-brain connectome-based computational modelling of meso-scale neural dynamics, including a novel and advanced methodology for single-subject TEP fitting and parameter estimation. We were able to identify at what point in time after the TMS pulse the outgoing and incoming connections of the stimulated site affect the TEP. Results indicate that recurrent activity begins to contribute to TEP measurements at the stimulated site from approximately 100ms post-stimulation, in line with the interpretation of these signal components as reflecting a recurrent network-level response. Further inspection and statistical analysis of estimated neurophysiological parameters additionally indicated an important role for the inhibitory population time constant in determining TEP excitability, identifying also key physiological contributors to the two principal dimensions of inter-subject variability in TEP waveforms. The novel discoveries and new software technologies introduced here should be of broad utility in basic and clinical neuroscience research.

Disclosures: **D. Momi:** None. **Z. Wang:** None. **J.D. Griffiths:** None.

Poster

667. Network Computation III

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Program #/Poster #: 667.13

Topic: I.06. Computation, Modeling, and Simulation

Support: Krembil Foundation Grant
Labatt Family Network
CAMH Discovery Fund

Title: A mathematical comparison of intracortical and corticothalamic models of EEG alpha rhythmogenesis

Authors: *S. BASTIAENS[1], J. D. GRIFFITHS[2];
[1]Inst. of Med. Sci., [2]Psychiatry, Univ. of Toronto, Toronto, ON, Canada

Abstract: The alpha rhythm (8-12Hz), which dominates EEG signals and is mainly observed during quiet wakefulness, plays a fundamental role in a wide range of cognitive processes. Abnormal alpha oscillations are also frequently observed in psychiatric and neurological conditions. However, even given the profound importance of alpha rhythms - both in terms of their undeniable prominence in empirical EEG data, and their implication across a broad range of phenomena across clinical and cognitive neuroscience, their mechanistic physiological basis and functional significance still remains unclear. Current theories on the generation of alpha rhythmic activity emphasize the importance of communication between various cortical and thalamic neural populations. This is represented by two prevailing types of neural population model (NPM) that posit alpha oscillations to be primarily driven by either: 1) recurrent activity and excitatory-inhibitory interactions within cortical column microcircuits; or 2) delayed inhibitory feedback within cortico-thalamocortical loops. Prominent examples of these two cortical and corticothalamic theories are the NPM models of Jansen & Rit (JR), and Robinson et al., respectively. There have been relatively few attempts to evaluate, compare in detail, and synthesize these theories. Therefore, in this study we developed an approach for comparing the dynamical repertoires and parameter space geometries of the two models. The rationale here is that even though the JR and Robinson models nominally describe differing neural populations and circuit motifs (e.g. intracortical, corticothalamic), their basic mathematical components, wiring structure, and excitatory/inhibitory sub-motifs can be meaningfully compared. Using this approach, we study their respective connectivity parameter spaces, through which we were able to identify similar dynamical patterns of excitatory and inhibitory population activity between the models. The influence of each feedback loop within the NPM circuit on the stability and frequency of oscillations is assessed. Finally, we formulate a novel three-dimensional reduction of the five-dimensional coupling strength parameter space of a JR-based model, which allows us to compactly summarize and visualize how the system dynamics change as a function of feedback loop gains. This work contributes to improving our mechanistic and theoretical understanding on candidate theories of alpha rhythmogenesis.

Disclosures: S. Bastiaens: None. **J.D. Griffiths:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

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Program #/Poster #: 667.14

Topic: I.06. Computation, Modeling, and Simulation

Support: Krembil Foundation Grant
Labatt Family Network
CAMH Discovery Fund
Branch Out Foundation Grant

Title: Accuracy and reliability of resting-state functional connectivity measurements from the Kernel Flow diffuse optical tomography (DOT) fNIRS system

Authors: *M. P. OVEISI[1], A. S. CLAPPISON[3], D. MOMI[4], J. D. GRIFFITHS[2];
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Abstract: Functional magnetic resonance imagining (fMRI) is a well-established non-invasive method for studying brain activity. However, due to its high cost of operation, limited availability, and restricted set of contexts in which it can be used, the applications of fMRI are limited. Fortunately, recent advances in neuroimaging technology have provided a promising new alternative to fMRI: functional diffuse optical tomography (fDOT). fDOT is a technique that utilizes high-density functional near infrared spectroscopy (fNIRS) to generate tomographic maps of haemodynamic signal fluctuations driven by the metabolic demands of neural activity. The Kernel Flow (KF) is a newly developed high-density fNIRS system that allows high-quality fDOT reconstructions while being a highly portable and relatively inexpensive device. To assess the capacity of the KF as a research-grade neuroimaging tool, we conducted a multiple test-retest experiment, where fNIRS recordings were obtained from 3 male participants across 20 10-minute sessions of eye-open resting state brain activity, spanning several days with variable within-day sampling density. Data were analyzed using a parcellation-based functional connectivity (FC) network approach in which we examined the reliability and consistency of resting-state fDOT connectivity patterns over hours, days, and across subjects. Additionally, the ability of the KF to detect functional resting state networks, as well as fingerprinting accuracy based on FC patterns was assessed. Results indicated high levels of test-retest reliability within and across subjects with a mean scan-to-scan Pearson correlation of R=0.47 (std=0.09) and R=0.39 (std=0.09), respectively. Moreover, results showed the KF is able to recover several typical fMRI-like data features such as modular separation of canonical brain networks and detection of cross-hemispheric coactivation of homologous brain regions. Lastly, fingerprinting results showed a 96% success rate in identification of subjects based on their FC patterns. We conclude that the new generation of high-density, high-quality, portable and affordable fNIRS fDOT systems, such as the KF, have major potential to improve and broaden functional brain data measurement in clinical and research settings. The first step towards this future is a comprehensive characterization of the quality and consistency of data from these devices, which has been our focus in this work.

Disclosures: M.P. Oveisi: None. **A.S. Clappison:** None. **D. Momi:** None. **J.D. Griffiths:** None.

Poster

667. Network Computation III

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Program #/Poster #: 667.15

Topic: I.06. Computation, Modeling, and Simulation

Support: Blue Brain Project
ETH Board

Title: Topological properties of a full-scale model of rat hippocampus CA1 and their functional implications

Authors: *K. KURBAN, H. MARKRAM, A. ROMANI;
EPFL, Blue Brain Project, Lausanne, Switzerland

Abstract: Hippocampus has a crucial role in the formation of declarative memories and navigation. Environmental cues from the cortical regions along with the current state of the brain are integrated, diagonalized, and redistributed within hippocampal subregions DG, CA3, CA2, and CA1. This phenomenon is heavily affected by the flow of information defined by the brain's connectome. Therefore, it is imperative to investigate the circuit structure and link it to the function.
In this study, we analyzed the topological features of our data-driven atlas-based rat CA1 model (Romani et al., 2022) that consists of 456K neurons and 820M intrinsic and 9.1B extrinsic synapses from upstream CA3 region. We observed that our model shows topological properties seen in other biological networks (e.g. small-world phenomenon, over- and under-expression of certain motifs, common neighbor bias). We selected a subset of these properties to further investigate the structure-function relationship. In particular, we manipulated the connectome in order to change the frequency of selected motifs, and tested how the manipulations affect the network behavior under several dynamics.

Disclosures: **K. Kurban:** None. **H. Markram:** None. **A. Romani:** None.

Poster

667. Network Computation III

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Topic: I.06. Computation, Modeling, and Simulation

Support: This study was supported by funding to the Blue Brain Project, a research center of the École polytechnique fédérale de Lausanne (EPFL), from the Swiss government's ETH Board of the Swiss Federal Institutes of Technology.

Title: Reconstructing visual thalamocortical input to a detailed, large-scale cortical network model

Authors: *H. DICTUS, A. ARNAUDON, J. HERTTUAINEN, G. IVASKA, A. ROMANI, H. MARKRAM;
Blue Brain Project, Geneve, Switzerland

Abstract: We reconstructed one hemisphere of mouse primary visual cortex and its thalamocortical input. The model consists of 322853 cells with unique detailed morphology across 56 morphological types, as well as 66473 LGN inputs without morphological detail. Connectivity, electrophysiology and synaptic properties are all constrained by the available experimental data. Thalamocortical connectivity is constrained according to receptive field properties associated with direction and orientation selectivity (Arkhipov et al. (2018), Lien and Scanziani (2013), Lien and Scanziani (2018)), synapse density estimates, and the morphologies of the target cells. We validate this with respect to the innervation patterns of different thalamocortical cell types and innervation probability of different cortical cell types. In particular, it has been shown that direction-selective and non-direction-selective thalamocortical afferents arborize in different layers of the cortex and connect with different cell populations (Cruz-Martín et al. (2014)). Additionally, it has been shown that the probability of thalamocortical innervation depends on a cell's gene expression (Ji et al. (2016)). We explore the extent to which the spatial distribution of thalamocortical afferents and cortical dendrites can explain this, thereby exploring the role of cellular morphology in this connectivity. Finally, we attempt to predict the innervation patterns for yet-uncharacterized cell types.
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Ji, Xu-ying, Brian Zingg, Lukas Mesik, Zhongju Xiao, Li I. Zhang, and Huizhong W. Tao. 2016. "Thalamocortical Innervation Pattern in Mouse Auditory and Visual Cortex: Laminar and Cell-Type Specificity." Cerebral Cortex 26 (6): 2612-25. https://doi.org/10.1093/cercor/bhv099.

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Poster

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Program #/Poster #: 667.17

Topic: I.06. Computation, Modeling, and Simulation

Title: Biophysically detailed single cell models of thalamic reticular nucleus neuron subpopulations

Authors: *P. LITVAK[1], N. HARTLEY[2], J. SIMKO[3], T. DAMART[1], W. VAN GEIT[1], R. J. KAST[4], Y. LI[2], V. G. LOPEZ HUERTA[5], S. K. SIMMONS[2], J. Z. LEVIN[2], H. MARKRAM[1], A. ROMANI[1], G. FENG[6], Z. FU[2], S. L. HILL[7];
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Abstract: The capacity of thalamic reticular nucleus (TRN) neurons to discharge in repeated bursts is a long known characteristic critical for the generation and maintenance of sleep rhythms. A recent report identified two TRN cell subpopulations with distinct electrophysiology characteristics strongly correlated with two distinct transcriptional programs, suggesting a molecular basis for rebound burst firing heterogeneity in the TRN (Li at al., 2020). While several computational models of reticular neurons have been developed to date, models capturing the heterogeneity of TRN firing dynamics are still lacking. Here, we present biophysically detailed single cell models representing two distinct TRN neuron subpopulations - Spp1 and Ecel1, constrained with experimental data from patch clamp recordings from Spp1-cre and Ecel1-cre mouse lines (Hartley et al., in preparation). We used a previously established multiobjective optimization algorithm to fit electrical models (Van Geit et al., 2016) constrained by features extracted from electrophysiology traces (N=118) and ion channels genetic expression data (N=36). The model neurons reproduced distinct electrophysiological signatures observed in experimental recordings of the neuronal subpopulations - differential low threshold rebound bursting and tonic discharges with distinct frequencies and attenuation at hyperpolarized and depolarized potentials. Furthermore, the model neurons' differential propensity to rebound burst could be explained by their relative difference in the T-type Ca2+ and Ca2+ activated small conductance K currents. This comprehensive modeling study offers insight into the differential contribution of TRN ionic mechanisms to rebound burst firing heterogeneity and provides a tool to further dissect the combined effect of different currents and their subcellular localization on TRN firing dynamics.

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Poster

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Topic: I.06. Computation, Modeling, and Simulation

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Title: Computational modeling of the unitary local field potential

Authors: *J. THARAYIL[1], M. W. REIMANN[1], E. NEUFELD[2], F. SCHUERMANN[1], A. DESTEXHE[3], H. MARKRAM[1];
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Abstract: The local field potential (LFP) refers to the voltage recorded by electrodes placed within neural tissue. The unitary LFP refers to the contribution of a single action potential, and its downstream effects, to the LFP signal; analyzing the unitary LFP may shed light on the micro-scale origins of the LFP signal. The unitary LFP also serves as a kernel, which when convolved with spike times from a phenomenological model, reproduces the LFP signal. Analysis of the unitary LFP in vivo has shown that the LFP primarily reflects inhibitory activity [1], but in vivo studies are limited by the difficulty of triggering action potentials in arbitrary cells, as well as uncertainty in the estimation of the unitary LFP. The unitary LFP depends on the morphology and physiology of the spiking cell and its postsynaptic targets, as well as on their positions relative to the recording electrode, and the connectivity and activity of the network. Computational modeling provides access to these parameters, allowing us to quantify their effect on the unitary LFP.
We evaluate the unitary LFP recorded from a cortical column in an in silico reconstruction of the rat somatosensory cortex, consisting of biophysically-detailed cells with realistic connectivity, based on the model in [2]. To calculate the unitary LFP from a selected cell, we run two identical simulations; for one of the two simulations, an additional synaptic input, corresponding to the action potential from the selected cell, is added. The contributions, from each cell, to the LFP from the two simulations are calculated and subtracted, yielding the contribution to the unitary LFP.
For each selected presynaptic cell, simulations are run for multiple LFP recording electrode positions. We quantify the impact of the additional action potential in terms of the amplitude and width of the difference in the extracellular potential, averaging over several trials. We identified non-trivial relationships between these metrics and the mean path length from the synapses on each postsynaptic cell to its soma, and the relative location of the efferent cell with respect to the electrode.
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[1] Teleńczuk B, et al. Local field potentials primarily reflect inhibitory neuron activity in human and monkey cortex. Sci Rep. 2017 Jan 11
[2] Markram H et al. Reconstruction and Simulation of Neocortical Microcircuitry. Cell. 2015 Oct 8

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Poster

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Title: A biological detailed model of a human cortical microcircuit

Authors: *N. BARROS-ZULAICA[1], V. SOOD[3], P. RAI[4], L. KANARI[5], A. ARNAUDON[7], Y. SHI[3], W. VAN GEIT[3], M. ZBILI[3], M. REVA[2], T. DAMART[3], J. DEFELIPE[8], R. BENAVIDES-PICCIONE[9], L. ALONSO-NANCLARES[10], C. P. DE KOCK[11], E. MERTENS[12], I. SEGEV[13], H. MARKRAM[6], M. W. REIMANN[14];
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Abstract: The neocortex is the brain area thought to differentiate humans most from other species because it gives us some unique abilities, as speaking. Although the human neocortex has been studied for many years since Ramon y Cajal started last century, very little is known about its anatomical structure and circuit functionality. One proven approach to speed up the process of understanding neurons and brain circuits that has been successfully used before, is the use of computational models and simulations. The approach combines and integrates the scattered data that is available into a coherent whole, allowing us to study their relations and dependencies. A complementary approach that aims to make the most out of the little data available for human, is to explicitly compare to and contrast it with better studied cortices from other species, such as rodents. In this study we built a first draft of a human cortical microcircuit following the approach described in Markram et al., 2015 to build a rat microcircuit. We collected, integrated, and analyzed data on human cortical anatomy and functionality that were available in the literature as well as produced by us, we also propose various strategies to overcome the missing data, such as generalizing or adapting data from other species. The goal of this model is to unravel human structural and functional cortical characteristics by comparing it with similar

rodent models. We found that human cells have more complex branching than rat cells, but lower bouton densities, and the number of connections between cells types is similar. We characterized the implications this has for the topological structure of connectivity in terms of robustness, degree distributions, symmetry and related measurements.

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Poster

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Title: Calcium-based plasticity and cell assemblies in a detailed, large-scale cortical network model in an in vivo-like state

Authors: *A. ECKER, D. EGAS SANTANDER, S. BOLAÑOS-PUCHET, J. B. ISBISTER, M. W. REIMANN;
EPFL - Blue Brain Project, EPFL - Blue Brain Project, Geneve, Switzerland

Abstract: The recent developments in experimental techniques have enabled simultaneous recordings of thousands of neurons. In particular, these techniques have made it possible to study the formation of functional cell assemblies. However, characterizing the evolution of synaptic connections and their plasticity within such assemblies remains challenging. To address this challenge, we developed a complementary simulation-based approach, using a detailed, large-scale cortical network model equipped with calcium-based functional plasticity of the synapses between excitatory cells. First, we detected functional cell assemblies from the stimulus-evoked spiking activity of 185’000 excitatory cells using a combination of recently published methods. Then, using algebraic topology (counting of directed simplices), we showed that the connectivity of cell assemblies is enriched with structural circuit motifs, thus linking their underlying structure to their co-firing "function". Last, by analyzing the evolution of the 320 million plastic synapses over 10 minutes of biological time, we found that strong but rare potentiation reorganized the network dynamics, while the more frequent but weaker depression kept it stable without the addition of homeostatic mechanisms. We observed significantly more potentiation in synapses between (temporally ordered) assemblies than within them, and higher than average

depression in synapses within assemblies, consistent with the experimental observation that stable structures cannot be potentiated further. In summary, we predict a highly organized structural connectivity underlying functional assemblies, small but frequent weakening of within-assembly synapses and strengthening of the ones connecting assemblies to each other.

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Poster

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Title: Modelling neuromodulation of neural microcircuits: effects of distinct cholinergic release modalities on synaptic transmission and network dynamics

Authors: *C. COLANGELO[1], A. ANTONIETTI[1], W. JI[1], H. MARKRAM[1], A. ROMANI[1], S. RAMASWAMY[2];
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Abstract: Acetylcholine (ACh) is one of the most studied neuromodulators in the rodent neocortex but, despite over a century of research, the fundamental biological organizing principles underlying cholinergic release have yet to be discovered. Simulation-based paradigms play an important role in testing scientific hypotheses and provide a useful framework to integrate the state-of-the-art knowledge and systematically explore previously uncharted territory (Markram et al 2015). To this end, we developed a multi-scale model of cholinergic innervation of rodent somatosensory cortex implementing either synaptic (ST) or volumetric transmission (VT) of ACh. The ST model entails a point-to-point, relatively fast transmission mode directly affecting the total conductance of the neuron thus modelling the effects of ACh on cellular excitability. The VT model, on the other hand, incorporates the main features of unwired transmission and was extended to include the effects of cholinergic release on synaptic connections that can be found in the volume of influence of ACh release (i.e., a sphere of radius 5 μm). The model enables the transmission modes to be combined in arbitrary proportions, thus permitting investigations of the relative contributions of these two transmission modalities. We find that the two modes of cholinergic release act in concert and reproduce established experimental findings such as a generalised increased in neuronal excitability (yet with morphology-specific peculiarities) and desynchronizing effects associated with a strong

reduction of delta oscillations. Moreover, we predict that the ACh-mediated decrease of glutammatergic synaptic release probability plays an important role in driving the network towards asynchrony. Overall, we show that our modelling framework can be used to study neuromodulatory release in the cortex, thus providing a new tool to study the complex interactions within different levels of brain organization.

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Poster

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Title: Looking alive: the quest for in vivo-like states in a detailed, large-scale cortical network model

Authors: *S. BOLAÑOS PUCHET, A. ECKER, J. B. ISBISTER, M. W. REIMANN;
Blue Brain Project, École Polytechnique Fédérale De Lausanne, Geneva, Switzerland

Abstract: Computational models of brain networks have been around for the past 25 years. A ubiquitous component of network models, no matter how simple or complex, is a mechanism that provides "external" or "background" inputs; these represent input from other parts of the brain not present in the model, due to reasons of scientific scope, practical limitations, or both. While often overlooked, these background inputs play the essential role of setting the baseline dynamical state of the network, from which evoked states develop. Having a network state that resembles *in vivo* activity is relevant for the comparison of simulation results with *in vivo* experimental measurements. Two commonly used mechanisms to provide background inputs are noisy current injection at the soma, and random activation of supplementary synapses. In the case of large-scale, data-driven, biophysically-detailed models, the former option is preferred, since synapses are computationally expensive and require hard-to-get experimental data for their parameterization. In this work, we study how to attain an *in vivo*-like network state in a detailed, large-scale cortical model, by injecting one of two different kinds of signals at the soma of single cells: a new type of shot noise process resembling random synaptic bombardment, or the well-known Ornstein-Uhlenbeck stochastic process. Firstly, we analyze single-cell behavior after injection of either current or conductance signals. Secondly, we describe the impact of injecting (properly weighted) signals to all cells in the network. Thirdly, we show that after careful tuning of input parameters, the network can exhibit per-layer spontaneous firing rates similar to what

has been measured *in vivo*. Finally, we discuss some general challenges of finding *in vivo*-like states in large-scale network models. To aid others in their own quests for relevant network states, we provide an open source implementation of the shot noise signal generation algorithm.

Disclosures: **S. Bolaños Puchet:** None. **A. Ecker:** None. **J.B. Isbister:** None. **M.W. Reimann:** None.

Poster

667. Network Computation III

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Topic: I.06. Computation, Modeling, and Simulation

Support: NIBIB: 5R01EB022889
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Title: HNN-core: an open-source Python interface to the Human Neocortical Neurosover (HNN) software for cellular and microcircuit interpretation of human MEG and EEG signals

Authors: *R. THORPE[1], M. JAS[2], N. TOLLEY[1], C. BAILEY[3], B. CALDWELL[1], M. A. SHERIF[4], M. HAMALAINEN[2], S. R. JONES[1];
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Abstract: The Human Neocortical Neurosolver (HNN) neural modeling software was created to provide a cell and microcircuit level interpretation of macroscale magneto- and electro-encephalography (M/EEG) signals. The foundation of HNN is a biophysically-detailed neocortical model, representing a patch of neocortex receiving thalamic and corticocortical drive. HNN was designed to simulate the time course of primary current dipoles and enables direct comparison, in nAm units, to source-localized M/EEG data, along with layer-specific cellular activity. HNN was created with a graphical user interface (GUI) and tutorials to teach users how to simulate common M/EEG signals, including event related potentials and low frequency brain rhythms. HNN-core (https://jonescompneurolab.github.io/hnn-core) is a Python package containing the core functionality of the HNN-GUI software, and is implemented with a clear application programming interface (API) instead of a GUI. Tutorials that mimic those available with the HNN-GUI (https://hnn.brown.edu/tutorials) include the simulation of ERPs and brain rhythms and describe how to employ a parameter optimization routine to identify a subset of parameters that can best reproduce a recorded current dipole evoked response. Additional HNN-core functions are available to modify the local network synaptic connectivity profiles, a feature not possible in the HNN-GUI. The Python interface also allows easy batch processing and

integration with M/EEG analysis pipelines based on MNE-Python; an example is available for median nerve evoked responses. Recent efforts have expanded HNN-core's ability to simultaneously simulate additional neurophysiological signals, including local field potentials (LFP) and current source density (CSD). Ongoing expansions include methods to compare model output to empirical data across these different modalities and to improve parameter estimation by constraining simulations to produce close agreement to more than one data type. These additional constraints will help minimize the number of possible parameter configurations that can accurately reproduce the empirical data.
HNN-core was created with best practices in open-source software to allow the computational and human neuroscience communities to understand and contribute to the development of the HNN software toolkit. The package is available to install with a single command on PyPI, is unit tested, adheres to modern Python style conventions, is extensively documented, and will help users understand and test hypotheses on the complex thalamocortical dynamics underlying neurophysiological signals emerging from the neocortex.

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Poster

667. Network Computation III

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Support: University of Miami
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Mcknight Doctoral Fellowship from the Florida Education Fund

Title: Laminar Specificity of the Auditory Awareness Negativity Under Multitone Masking: A Biophysical Modeling Study

Authors: *C. FERNANDEZ PUJOL[1], A. R. DYKSTRA[1], E. G. BLUNDON[2];
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Abstract: Electroencephalography and magnetoencephalography are excellent mediums for capturing human neural activity on a millisecond time scale, yet little is known about their underlying laminar and biophysical basis. Here, we used a reduced but realistic cortical circuit model -Human Neocortical Neurosolver (HNN)- to shed light on the laminar specificity of brain responses associated with auditory conscious perception under multitone masking. HNN provides a canonical model of a neocortical column circuit, including both excitatory pyramidal and inhibitory basket neurons in layers II/III and layer V. We found that the difference in event-related responses between perceived and unperceived target tones could be accounted for by

additional input to supragranular layers arriving from either the non-lemniscal thalamus or cortico-cortical feedback connections. Layer-specific spiking activity of the circuit revealed that the additional negative-going peak that was present for detected but not undetected target tones was accompanied by increased firing of layer-V pyramidal neurons. These results are consistent with current cellular models of conscious processing and help bridge the gap between the macro and micro levels of analysis of perception-related brain activity.

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Poster

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Title: Adaptation to Repetitive Stimuli Associated with Increased Gamma Oscillations in an Auditory Cortex Computer Model

Authors: *M. W. M. ELSAYED[1,2], W. W. LYTTON[3], M. A. SHERIF[4];
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Abstract: Repetitive sensory stimuli produce reduced neuronal firing, known as adaptation. Despite reduced firing, perception is maintained. Earlier evidence postulated that simultaneous increase in gamma synchrony is responsible for maintained perception. To examine possible mechanisms underlying increased gamma synchrony with reduced neuronal firing, we used the human neocortical neurosolver (HNN), a validated computer model of primary sensory neocortical microcircuitry that replicates neocortical activity following stimuli by simulating feedforward and feedback inputs.
The HNN model consists of 200 multicompartmental pyramidal neurons (PN) and 70 inhibitory basket interneurons arranged in equal distribution in supragranular and infragranular layers (layers 2/3 and 5 respectively). We delivered four stimuli to the network with different interstimulus intervals: 50, 100, 150, 200, 300, and 400 ms. Each of the stimulus consisted of a feedforward drive arriving at the soma, followed by a feedback drive targeting the distal apical dendrites, then another drive targeting pyramidal neuronal soma, a pattern that replicates the P50, N100, and P200 event-related potentials.
With 50 and 100 ms interstimulus interval of repetitive stimuli, and to a lesser extent with 150 and 200 ms, we found reduced firing of layer 5 pyramidal neurons, reflecting adaptation. At an interstimulus interval of 300 ms or more, there were no changes in the firing rate of either layer

2/3 or layer 5 pyramidal neurons. Interestingly, with an interstimulus interval of 100 ms (and to a lesser extent with 150 ms), we found transient episodes of emergent gamma synchronized activity of layer 2 pyramidal and basket interneurons. These gamma oscillations were produced by a mechanism of layer 2 pyramidal neurons and basket interneurons creating a more sustained gamma rhythm.
We describe a mechanism by which adaptation is associated with increased gamma oscillations arising from distinct laminar dynamics. We will next examine how interlaminar interactions and drives result in this phenomenon.

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Poster

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Title: Simulation based inference in the Human Neocortical Neurosolver software reveals a calcium-mediated decoupling between spiking and field activity underlying macroscale M/EEG event related potentials

Authors: *N. M. TOLLEY[1], P. L. C. RODRIGUES[2], S. PUGLIESE[1], A. GRAMFORT[3], S. R. JONES[1];
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Abstract: Event related potentials (ERP) measured by magneto- and electroencephalography (M/EEG) are a noninvasive and fundamental measure of neural activity that can be used to distinguish cognitive and disease states. Understanding the cellular and circuit-level origins of ERP generation is critical to deriving theories on sensory information processing, and to develop therapeutics based on neuropathological ERPs. Computational modeling with the Human Neocortical Neurosolver (HNN; Neymotin et al., 2020) is a powerful technique to study the neural mechanisms of ERPs, and demonstrated that they can be generated by a sequence of laminar specific excitatory inputs to the cortical column. However, previous methods used with HNN were designed to find a single optimized set of parameters that account for a specific ERP

waveform. An exploration of the broader distribution of parameters that account for a given ERP, particularly in relation to cellular properties such as spiking and calcium dynamics, has not been fully performed. To robustly characterize ERP generation at multiple scales, we applied simulation based inference (SBI), a deep learning based Bayesian inference framework (Cranmer et al., 2020), to HNN. The main utility of SBI is a posterior estimate of the parameter distributions and activity patterns that can account for specific ERP waveforms.
We examined 3 forms of neural activity associated with tactile evoked ERPs: 1) the current dipole, which underlies M/EEG, 2) local field potentials (LFP), and 3) cell-specific spiking. By varying the strength and timing of the laminar specific inputs, as well as dendritic calcium currents of pyramidal neurons, we revealed that spiking activity can be highly decoupled from the associated ERP waveform. Specific ERPs could be equivalently produced by a large variety of spiking patterns, while others by a limited set. SBI revealed that the level of spike-ERP decoupling was dependent on a strong interaction between dendritic calcium and laminar specific inputs. In future work, we will assess how this interaction impacts ERP features associated with perceptibility of sensory stimuli.

Disclosures: **N.M. Tolley:** None. **P.L.C. Rodrigues:** None. **S. Pugliese:** None. **A. Gramfort:** None. **S.R. Jones:** None.

Poster

667. Network Computation III

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 667.27

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R01 NS102201
NSF CAREER 1752355
Roy J. Carver Charitable Trust Research Program of Excellence

Title: Biophysical circuit-mechanisms of the stop-signal P3 during action-stopping

Authors: *D. A. DIESBURG[1], J. R. WESSEL[2], S. R. JONES[1];
[1]Dept. of Neurosci., Brown Univ., Providence, RI; [2]Dept. of Psychological and Brain Sci. / Dept. of Neurol., Univ. of Iowa, Iowa City, IA

Abstract: The horse race model of motor inhibition is a theoretical account of inhibitory control in the stop-signal task (SST). It suggests that action-stopping results from a race between a go- and stop-process, with successful stops resulting from a faster stop-process or slower go-process. It has been proposed that the EEG-measured frontocentral-P3 (FC-P3) event-related potential (ERP) may reflect the activity of this underlying stop-process because of its earlier onset in successful compared to failed stops. Although characteristics of the FC-P3, such as onset time, have been linked to motor inhibition in the SST, investigating whether its *mechanisms* align with the race model is challenging due to the complex dynamics that underlie evoked cortical activity.

The Human Neocortical Neurosolver (HNN) software (Neymotin et al., 2020), whose foundation is a biophysical model of canonical neocortical column architecture, can be used to probe circuit mechanisms of the FC-P3 ERP. We postulate that neural mechanisms of the P3 will onset *earlier* in successful vs. failed stops.
We examined FC-P3 ERPs from 234 EEG datasets collected during a SST in healthy participants, and applied HNN to develop and test predictions on the circuit mechanisms underlying ERPs observed during successful and failed stops. We observed that the FC-P3 onsets and peaks earlier in successful compared to failed stop conditions, with successful and failed stop P3 peaks observed at 316ms and 360ms on average, respectively. The HNN model predicts the FC-P3 waveform deflection is generated by excitatory feedforward thalamocortical drive that effectively activates the proximal pyramidal neuron dendrites at ~300ms. Consistent with our hypothesis, the observed earlier P3 onset time in successful compared to failed stop conditions can be reproduced in the model by adjusting the arrival time of feedforward thalamic drive to the cortex to be ~20ms *earlier* in successful stops trials compared to simulated failed stop trials. Importantly, the effective strength of the drive was unchanged across conditions. We tested several alternative hypotheses in the model, including testing if reduced synaptic weights or changes in number of spikes providing the feedforward drive could account for the observed FC-P3 difference, and found that changes in the arrival time most accurately reflected the empirical data.
These results suggest a race model account of the FC-P3 aligns with cortical dynamics during action-stopping in the SST. Moreover, biophysical modeling with HNN can help interrogate theoretical accounts of ERPs and their relation to underlying neural mechanisms.

Disclosures: D.A. Diesburg: None. **J.R. Wessel:** None. **S.R. Jones:** None.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.01

Topic: I.06. Computation, Modeling, and Simulation

Support: RF1 AG065675
R01 AG067153

Title: Nonnegative regression mitigates background expression in mouse brain spatial transcriptomics (MERFISH) data

Authors: *A. GELBER[1], K. G. JOHNSTON[3], B. T. BERACKEY[4], L. CHEN[8], K. TRAN[5], L. TRAN[2], K. N. GREEN[9], Q. NIE[6], G. MACGREGOR[7], Z. TAN[10], X. XU[11], E. A. MUKAMEL[12]; [1]Bioengineering, [2]Cognitive Sci., UCSD, San Diego, CA; [3]Mathematics, Univ. of California, Irvine, Irvine, CA, CA; [4]Biomed. Engin., Univ. of California, Irvine, Irvime, CA; [5]Neurobio. and Behavior, [6]Mathematics, [7]Biol., Univ. of California, Irvine, Irvine, CA; [8]Dept. of Anat. and neurobiology, Univ. of California Irvine, Irvine, CA; [9]Neurobio. and Behavior, Univ. of

California, Irvine, CA; [10]Inst. for Memory Impairments and Neurolog. Disorders, Univ. California Irvine, Irvine, CA; [11]Anat. and Neurobio., Univ. California, Irvine, Irvine, CA; [12]Cognitive Sci., Univ. of California San Diego, La Jolla, CA

Abstract: Spatial transcriptomics based on multiplexed single-molecule fluorescence in situ hybridization (e.g., MERFISH) can assay hundreds of RNA species in situ with single-molecule resolution. This technology can identify and localize brain cell types, and is well suited to investigating cellular and molecular responses to disease and injury. While computational methods can segment cells and assign transcripts to their cells of origin, the complex nature of brain tissue leads to a high rate of error in this task, even using state-of-the-art segmentation approaches. We addressed this issue by analyzing a MERFISH dataset assaying 150 RNA species in 4 coronal sections of the mouse brain. We observed that highly expressed genes generate many dendritic and axonal mRNA transcripts which are detected outside the segmented cell body. These molecules may be assigned to an incorrect cell when their location falls near a cell body, preventing reliable optical resolution of the precise cell boundary. Such events contaminate estimated gene expression and lead to errors in differential gene expression analysis. Whereas background estimation and subtraction has been addressed for snRNA-seq data (Fleming et al. 2019), no such method has been developed for spatial transcriptomics. To correct for erroneously assigned transcripts, we propose a method to decompose observed expression vectors into a linear combination of true expression and local background expression. Non-negative LASSO regression was used to decompose observed expression vectors into linear combinations of individual gene contributions and local background vectors. Because the basis for this decomposition is over-complete, an L1 penalty term was used to enforce sparseness in the estimated expression of individual genes. This effectively performs variable selection, setting the estimated expression for some genes to zero if their counts are well-approximated by the local background. Notably, this approach is computationally efficient and can be applied to hundreds of thousands or millions of cells with modest numerical resources. We show that our approach has the desired effect of substantially reducing known contaminants while minimally affecting clustering and low dimensional embedding. Additionally, differential expression analysis on the background-removed dataset yields fewer erroneous results, assessed by comparing significance of known markers in the raw and background-removed data. Moreover, the method is flexible and allows the user to select markers to be explicitly removed from the true expression basis and adjust background removal strength by adjusting penalty tuning parameters.

Disclosures: **A. Gelber:** None. **K.G. Johnston:** None. **B.T. Berackey:** None. **L. Chen:** None. **K. Tran:** None. **L. Tran:** None. **K.N. Green:** None. **Q. Nie:** None. **G. MacGregor:** None. **Z. Tan:** None. **X. Xu:** None. **E.A. Mukamel:** None.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.02

Topic: I.07. Data Analysis and Statistics

Support: Schmidt Family Foundation

Title: Sims: a minimal-code tool for cell type classification from single-cell transcriptomic data and its application in neuroscience

Authors: *J. LEHRER, D. HAUSSLER, M. TEODORESCU, M. MOSTAJO-RADJI, V. JONSSON;
UC Santa Cruz, Santa Cruz, CA

Abstract: Experiments in single-cell transcriptomics have become increasingly large and complex. The capacity to readily collect these datasets has contributed to unprecedented biological insight - and concurrently, a data deluge. In principle, single-cell RNA-seq atlases should serve as a reference for automation, yet in practice classification algorithms have lacked the capacity to do so. Tasks such as cell annotation and cell state characterization increasingly necessitate automation, and while data driven methods aimed at inferring cell state from transcriptomic data are currently in development, a focus on robustness, scalability and interpretability are paramount.
We present SIMS: an end-to-end machine learning pipeline for discrete morphological prediction of single-cell data with minimal code and high classification accuracy. Using a transformer-based architecture with sequential attention layers for interpretable classifications, SIMS takes an expression matrix with associated labels and learns a mapping between transcriptome and cell type. This mapping can then be used to automatically infer cell types in new single-cell data. By performing several ablative studies across multiple experimental conditions we show that only a subset of 4k cells are needed to build an accurate model. Additionally, we show that SIMS performs well between tissue samples and outperforms scANVI, one of the most popular cell type classification algorithms on several benchmark datasets. We also describe and implement how classification outputs can be directly characterized from the raw input transcriptome, allowing for interpretability at the level of individual samples, class, and globally.
We applied SIMS to classify existing datasets of the brain and retina. Moreover, we use our architecture for fast classification of stem-cell derived neurons originating from 2D cultures as well as cortical organoids.
We present a new, easy-to-use tool for cell type classification from single-cell transcriptomic data.

Disclosures: **J. Lehrer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor in provisional patent related to this work. **D. Haussler:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor in provisional patent related to this work. **M. Teodorescu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor in provisional patent related to this work. **M. Mostajo-Radji:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor in provisional patent related to this work. **V. Jonsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor in provisional patent related to this work.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.03

Topic: I.07. Data Analysis and Statistics

Support: R35 GM119831

Title: Sex-based snRNA-seq multiplexing to increase rigor and reproducibility and decrease experimental cost

Authors: *N. CHU, K. CICHEWICZ, C. POLLARD, A. NORD;
Ctr. for Neurosci., Univ. of California Davis, Davis, CA

Abstract: Single cell or single nuclei transcriptomic profiling (hereafter snRNA-seq) has emerged as the dominant method to comprehensively profile cell-type specific molecular states in the brain. As snRNA-seq is expensive and prone to batch effects, there is a necessary trade-off between cost and inclusion of replicates. This is compounded where the goal is detection of phenotypes that differ between conditions, treatments, or sex, where changes can be subtle and biological replicates are critical. To mitigate batch effects, reduce costs, and enable investigation of sex-specific effects, we developed a probabilistic approach to distinguish male and female samples pooled into one sequencing run. This method is complementary with hashing of lipid-based barcoding, but does not require incorporation of molecular labels during sample preparation. We use sex-linked gene expression patterns modeled at the cell-type (i.e. cluster) level to make a probabilistic assignment of cell sex accounting for experimental background. We demonstrate proof of concept by analyzing snRNA-seq data from adult and neonatal mouse brain. Samples were collected from sex balanced cohorts of mutant and wildtype littermates across several mutant mouse lines. As a comparison and control, we found that sex-based sample assignment shows agreement between CMOs, and can help deconvolute situations where multiple CMOs are detected. Limitations of this method are the potential sparse detection of relevant sex-linked transcripts and requirement for correction of sampling bias between male and female transcripts that results in higher probability calls for female cells. Further refinement of this approach to snRNA-seq will address the barrier of cost in generating large sets of RNA seq data and improve rigor and reproducibility of these experiments.

Disclosures: N. Chu: None. **K. Cichewicz:** None. **C. Pollard:** None. **A. Nord:** None.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.04

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant award 1R43DA056084-01

Title: Artificial intelligence driven prediction of the blood-brain barrier permeability using drug structural data

Authors: *M. KHOTIMCHENKO;
VeriSIM Life, San Francisco, CA

Abstract: Brain distribution is a key pharmacokinetic property of therapeutics targeted for central nervous system disorders. The blood-brain barrier (BBB) plays a critical role in controlling the influx and efflux of biological substances essential for the brain's metabolic activity as well as neuronal function. Due to the complexity of BBB, drug transport into the brain can barely be calculated from lipophilicity and dissociation constant, thus making drug development procedures more expensive and complicated. Recently, artificial intelligence (AI) and machine learning (ML) approaches have been proposed for the prediction of brain drug disposition using chemical properties and PK parameters. We have developed a two-component AI/ML model that can predict drug BBB permeability with an accuracy higher than 90% using only structural data i.e. SMILES string. It is composed of a classification model that predicts drug dichotomic capacities of either permeation or non-permeation of the BBB; and a regression model predicting qualitative permeability. The training set for the classification model contained more than seven thousand compounds, whereas the regression model was trained across a dataset containing around one thousand drugs. A validated model was implemented to determine compounds with the best drug disposition properties from a database with 44 thousand virtual compounds. Experimental studies confirmed accuracy of predicted data indicating the blood-brain ratio. Our studies suggested that AI/ML model can accelerate the drug development process showing a favorable PK profile for drugs intended for the treatment of central nervous system disorders.

Disclosures: **M. Khotimchenko:** A. Employment/Salary (full or part-time):; VeriSIM Life. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VeriSIM Life.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.05

Topic: I.06. Computation, Modeling, and Simulation

Title: Evaluating u-net mouse brain extraction for multi-site and longitudinal preclinical stroke imaging

Authors: *E. TARAKCI[1], J. MANDEVILLE[3], F. HYDER[6], B. G. SANGANAHALLI[8], D. R. THEDENS[9], S. HUANG[10], A. ARBAB[11], A. BIBIC[12], J. MIHAILOVIC[7], A. MORAIS[4], J. LAMB[13], K. NAGARKATTI[13], M. DINITZ[15], A. ROGATKO[15], A. W. TOGA[2], P. LYDEN[14], C. AYATA[5], R. CABEEN[1];
[2]USC Stevens Neuroimaging and Informatics Inst., [1]USC, Los Angeles, CA; [4]Dept. of Radiology, Dept. of Neurol., [3]Harvard Med. Sch., Boston, MA; [5]Dept. of Radiology, Dept. of Neurology,, Harvard Med. Sch., Charlestown, MA; [7]Departments of Radiology and Biomed. Imaging, Departments of Biomed. Engin., [6]Yale Univ., New Haven, CT; [8]Diagnos. Radiology, Yale Univ. Sch. of Med., New Haven, CT; [9]Dept. of Epidemiology, Univ. of Iowa, Iowa City, IA; [10]Dept. of Diagnos. and Interventional Imaging, Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [11]Med. Col. of Georgia, Augusta Univ., Augysta, GA; [12]Dept. of Anesthesiol. and Critical Care Med., Johns Hopkins Univ., Baltimore, MD; [13]Dept. of Physiol. and Neurosci., [14]Neurol., Zilkha Neurogenetic Inst., Los Angeles, CA; [15]Biostatistics and Bioinformatics Res. Center, Samuel Oschin Comprehensive Cancer Ctr., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Rodent stroke models are important for evaluating treatments and understanding the pathophysiology and behavioral changes of brain ischemia, and magnetic resonance imaging (MRI) is a valuable tool for measuring outcome in preclinical studies. Brain extraction is an essential first step in most neuroimaging pipelines; however, it can be challenging in the presence of severe pathology and when dataset quality is highly variable. Convolutional neural networks (CNNs) can improve accuracy and reduce operator time, facilitating high throughput preclinical studies. As part of an ongoing multi-site preclinical stroke imaging study, we developed a deep-learning mouse brain extraction tool by using a U-net CNN. While previous studies have evaluated U-net architectures, we sought to evaluate their practical performance across data types, field strengths, and acquisition setups. We ask how performance is affected with data across six imaging centers, two time points after experimental stroke (2 days and 30 days), and across four MRI contrasts. We trained, validated, and tested a typical U-net model on 240 4-channel multimodal MRI datasets including quantitative multi-echo T2 and apparent diffusivity coefficient (ADC) maps, and performed qualitative evaluation with a large preclinical stroke database (N=1,368). In addition, we trained a total of 8 U-net models, varying across sites, contrasts, and single vs. multi-channel inputs and used Dice scores to evaluate segmentation accuracy against the ground truth results from the traditional segmentation method. We consistently found high accuracy and ability of the U-net architecture to generalize performance in a range of 95-96% accuracy, with only modest reductions in performance based on lower fidelity imaging hardware and brain pathology across all 8 models. We found that the single-channel model of the U-net is nearly as robust as the multi-channel model which allows for flexibility in data collection. The model also performed at 95% accuracy for out-of-sample sites, suggesting that the U-net model accommodates the addition of new sites without further training or a major reduction in accuracy – a beneficial feature for multi-site studies. The he flexibility of the U-net could be further assessed by testing the U-Net's transferability to rats and evaluating the speed of this approach on various hardware available to labs (ie. GPU vs CPU). This work can help inform the design of future preclinical rodent imaging studies and improve their scalability and reliability.

Disclosures: E. Tarakci: None. **J. Mandeville:** None. **F. Hyder:** None. **B.G. Sanganahalli:** None. **D.R. Thedens:** None. **S. Huang:** None. **A. Arbab:** None. **A. Bibic:** None. **J. Mihailovic:**

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Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.06

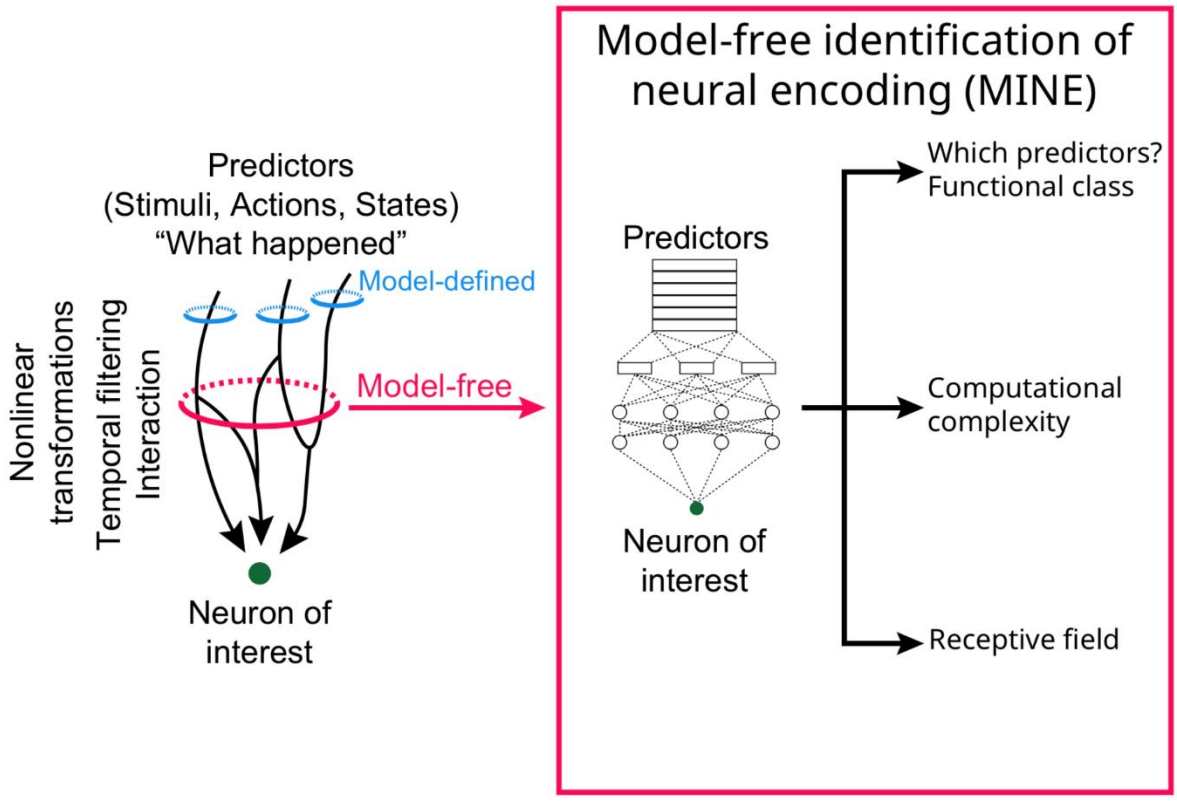
Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant 1R01NS123887-01

Title: A model-free approach to link neural activity to behavioral tasks

Authors: J. COSTABILE, **K. A. BALAKRISHNAN**, S. SCHWINN, *M. HAESEMEYER; Dept. of Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Contemporary neuroscience approaches produce large datasets of neural activity in behaving animals. To generate insight from such data, it is often desired to identify neurons with activity related to the behavior at hand. A common approach to this problem is to intuit a functional form ("a model") by which the activity might be related to aspects of the task or state of the animal. However, brains aren't engineered solutions to a well-defined problem but arose through selective pressure acting on random variation. It is therefore unclear how well neural activity can be captured by a well-defined function chosen as a model by the experimenter. Here we developed an approach, "Model-free identification of neural encoding (MINE)", using small convolutional neural networks to learn both parameters and an implicit model relating aspects of a task to single neuron activity. Although flexible, artificial neural networks are hard to interpret. We therefore use network decomposition and approaches from explainable machine learning to form a post-hoc understanding of the mapping from task to activity encapsulated by the networks. We apply this approach to a published cortical mouse widefield imaging dataset as well as experiments we designed to probe thermoregulatory circuits in larval zebrafish. Here, MINE allowed us to characterize neurons encoding specific stimulus and behavior features. We also identified a class of neurons that integrate thermosensory and behavioral information, which eluded us previously when using clustering and regression-based approaches.



Disclosures: **J. Costabile:** None. **K.A. Balakrishnan:** None. **S. Schwinn:** None. **M. Haesemeyer:** None.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.07

Topic: I.06. Computation, Modeling, and Simulation

Title: Automated portable stereotaxic device

Authors: ***R. BELTRAN-RAMIREZ**[1], J. MARTINEZ-MENDOZA[2], M. MACIEL ARELLANO[2], V. LARIOS-ROSILLO[2], J. ORIZAGA TREJO[2], J. DOMÍNGUEZ RAMÍREZ[2], X. BECERRA GONZÁLEZ[2], X. JIMENEZ ROMAN[2];
[1]Univ. de Guadalajara, Zapopan, Mexico; [2]Univ. De Guadalajara, Zapopan, Mexico

Abstract: AUTOMATED PORTABLE STEREOTAXIC DEVICE The present invention describes an automated portable stereotaxic device that has a light and easily transportable structure, which allows interventions to be carried out anywhere, in addition to its spherical crest shape, it is possible to maintain safety inside the device, another One of the characteristics of the

present invention is that it can be operated remotely from any type of intelligent device, allowing contamination to be eliminated from the outside to perform surgeries free of infection, in addition to the fact that by remote programming it is possible to establish the parameters operation of the device, including the extraction of the plate to place the animal to be operated on, its introduction and the operation of the intervention instruments, performing the surgery automatically. The automated portable stereotaxic device also has an anesthetic solution container that is manipulated from the outside, which allows the animal inside the device to be sedated without having contact with it. It also has the characteristic of monitoring in real time the vital signs of the animal that is in the intervention.

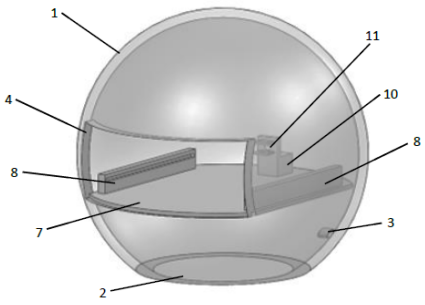
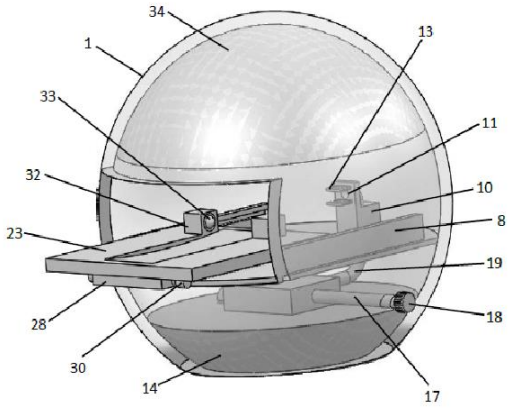


FIGURA 1



Disclosures: **R. Beltran-Ramirez:** None. **J. Martinez-Mendoza:** None. **M. Maciel Arellano:** None. **V. Larios-Rosillo:** None. **J. Orizaga Trejo:** None. **J. Domínguez ramírez:** None. **X. Becerra González:** None. **X. Jimenez roman:** None.

Poster

668. Computational Tools for Experimental Studies

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Program #/Poster #: 668.08

Topic: I.06. Computation, Modeling, and Simulation

Support: 2022 Joint Research Project of Institutes of Science and Technology

Title: In-silico Analysis Framework of Transcranial Electrical Stimulation (tES) for Animal Study

Authors: J. LEE[1], Z. RIEU[1], S. JIN[2], Y. GONG[2], Y. JUN[2], J. YONG[1], H. CHO[2], ***D. KIM**[1]; [1]NEUROPHET, Inc., Seoul, Korea, Republic of; [2]Biomed. Engin., UNIST, Ulsan, Korea, Republic of

Abstract: The mechanism of transcranial electrical stimulation (tES) is still not fully understood although tES is widely used to investigate brain functions and to treat neurological/psychiatric disorders in humans. The laboratory study with animal model is essential to study the mechanism because a simultaneous, invasive recording while a tES application is relatively easier. However, the resources for the required facilities, experts, and maintenances are regarded as big burdens, which can be increased with various tES dose parameters to test. Simulation-based approach could help reduce the burden as well as effectively design a study. Therefore, we developed a GUI-based software to simulate tES-induced electric field (E-field) in the rat. This software enables to predict and analyze E-field distributions within a realistic 3D model of a rat under given tES parameters: tES type (tDCS, tACS), stimulation intensity, and electrode shape/size/position/number. Our standalone software, coded in C++, does not require any coding or library. tES parameters are adjustable via the GUI. Three electrode shapes are available: disc, ring, and rectangle with a user-defined size. Electrode can be positioned by inputting a coordinate or clicking in the 3D model. As an input, a paired T2-weighted MRI and CT scan of a rat's whole brain is required. The entire pipeline consists of three steps: tissue segmentation, 3D modeling, and simulation. Each step is easily done with the button on the GUI. First, the following five segmentation labels are generated: gray matter (GM), white matter (WM), CSF, bone, and soft tissue. The GM, WM, and CSF labels are generated with image registration of T2w MRI on the Fischer 344 template, while the bone and soft tissue labels are generated by CT scan using the Hounsfield unit. Next, a realistic 3D volumetric model is created from the labelled data as follows. Tetrahedron meshes for each tissue are produced. Then, surface meshes are created between different tissues. A volume conductor model is made by assigning an isotropic electrical conductivity to each tissue: GM=0.276, WM=0.126, CSF=1.65, bone=0.01, and soft tissue=0.465 S/m, which are editable. Finally, for the simulation, finite element method is applied to solve quasi-static Maxwell's equations. The resultant outcomes are an electric field vector and strength (V/m). Those outcomes can be visualized in the MRI or 3D model and can be exported. In addition, an ROI-based analysis of the E-field is available. We believe that our new

software will facilitate animal model-based study of tES by enabling simulation-based study design and reducing the time and cost for laboratory animals.

Disclosures: **J. Lee:** None. **Z. Rieu:** None. **S. Jin:** None. **Y. Gong:** None. **Y. Jun:** None. **J. Yong:** None. **H. Cho:** None. **D. Kim:** None.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.09

Topic: I.06. Computation, Modeling, and Simulation

Support: American Heart Association Collaborative Sciences Award #18CSA33990385

Title: Automated data processing tool for continuous blood pressure neuromodulation

Authors: *M. GARZA, K. ROMERO, D. LLOYD, M. A. GONZÁLEZ-GONZÁLEZ, M. I. ROMERO-ORTEGA;
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Abstract: Data analysis obtained from biological systems is critical for understanding complicated physiological mechanisms and often is time-consuming, leading to missed important experimental aspects. Continuous blood pressure (BP) measurements may provide insights to understand cardiovascular mechanisms such as hypertension or circadian control of blood pressure. However, it requires data processing tools that help optimize data analysis. We recently reported that electrical modulation of the deep peroneal nerve (DPN) using a wired microchannel multielectrode elicited a vascular depressor response in spontaneously hypertensive rats (SHR, n=9, $p<0.0001$). Chronic evaluation of the DPN neuromodulation in hypertension using a novel miniaturized wireless microchannel neuro-clip (wNClip) electrode was done using a BP telemetry system generates large amounts of data, which across several time points and conditions complicated the rigorous analysis. We developed an automated program to configure raw pressure data collected from the implantable wireless BP telemetry device (DSI). We used the standard ‘Scipy’ and ‘Numpy’ libraries in Python 3.8.5 for all computations and Tk interface for the GUI (graphical user interface). Our novel application contains a GUI to use on large amounts of data (460 files in our case). It processes the raw physiological data obtained from continuous telemetric measurements in awake animals, and automatically organizes the data by categories such as subject, type, date, and time, and synchronizes experiments with real-time events. The daily files are processed into a singular CSV file with a label column that assigns a number indicating if the data was from prior to, during, or post-treatment. The program then generates daily mean arterial blood pressure figures with color coding dependent on the treatment label. A subroutine removes segments with aberrant readings due to events such as animal handling. The data points are averaged, and a data file with each row as a different day is generated. Automating the initial processing helps to

standardize data and vastly speed up the analysis of this data from a few days to less than an hour. By enabling fast and automated data processing we can ensure efficacy of neuromodulation treatments with rigor and reproducibility while reducing the analysis time from several hours to approximately thirty minutes.

Disclosures: **M. Garza:** None. **K. Romero:** None. **D. Lloyd:** None. **M.A. González-González:** None. **M.I. Romero-Ortega:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RBI Medical.

Poster

668. Computational Tools for Experimental Studies

Location: SDCC Halls B-H

Time: Wednesday, November 16, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 668.10

Topic: I.07. Data Analysis and Statistics

Support: PR19-CR-P2 BioSUP INAIL

Title: Decoding the bladder fullness from intraneural pudendal nerve signals using a machine learning algorithm

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Abstract: Lower urinary tract dysfunctions can result in impaired storage, including bladder sensation and voiding with possible negative effects on urinary continence and quality of life. Previous research has attempted to monitor the bladder function through feline pudendal nerve using cuffs and Utah Slanted Electrode Array but recordings lacked selectivity and long term stability.
Here, pudendal nerve activity of pigs was recorded using 16-channel transverse intrafascicular multi-electrodes (TIMEs) under empty and full bladder conditions (defined as the volume of saline infused until urine leakage). We performed surgical exposure of the left pudendal nerve in n=2 anesthetized male farm pigs (30-32kg, 3-4 months old) by using a transgluteal approach. We

implanted one TIME in the first animal and two TIMEs distal and proximal in the second animal. We acquired neural signals in the empty and full bladder conditions for 5 minutes, respectively (full bladder volumes 550ml and 700 ml for the two animals, respectively). We decoded empty and full bladder conditions using a decoding algorithm suitable for band passed [1000,6000] Hz intraneural windowed (100 ms) signals. We performed feature extraction by estimating for each recording channel: variance, kurtosis, skewness, wavelength, mean absolute value, peak to peak amplitude, and maximum value for rectified signal. We optimized a k-NN classifier using a Nested Cross Validation scheme with 5 outer and 3 inner folds.
We classified empty and full bladder conditions with high accuracy values i.e., 87 (mean) ± 3 (SD)% (n=5 k-fold) and 82 ± 1% on the first and second animal, respectively. In the second animal, we performed pudendal nerve transection between the proximal and distal electrodes, and we compared the accuracies obtained by using proximal and distal electrodes in intact and transected nerve conditions. For the proximal electrode, we found a significant reduction in decoding performances ($p<0.01$, Wilcoxon rank-sum test, n=5 k-fold) by achieving 77 ± 1 % and 64 ± 3 % accuracy values in intact and transected nerve conditions, respectively. This result could be explained by the absence of afferent sensory fibers conveying information on bladder fullness. A significant accuracy increase was obtained by using the distal electrode 77 ± 3 % and 90 ± 2 % (intact and transected, respectively, $p<0.01$ Wilcoxon rank sum test, n=5 k-fold), probably due to denoising of afferent sensory signals by filtering out uninformative efferent motor fibers.
Our results could be a step towards a closed-loop system for restoring voiding control and bladder sensation through a bidirectional intraneural interface implanted in the pudendal nerve.

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Poster

668. Computational Tools for Experimental Studies

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Program #/Poster #: 668.11

Topic: I.07. Data Analysis and Statistics

Title: Automated Extraction of Heart Rate VariabilityAutomated Extraction of Heart Rate Variability Data from Magnetoenecephalography ScansData from Magnetoencephalography Scan

Authors: *W. FLOOD[1], R. GODWIN[2];
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Abstract: Motivation/Problem statement: Heart rate variability is emerging as a viable biomarker for insight into the autonomic nervous system, revealing the cardiovascular

implications of numerous pathologies. The purpose of this investigation was to develop a method to retroactively extract cardiovascular data from magnetoencephalography (MEG) scans and then to subsequently quantify this data to elucidate subject-specific heart rate variability. **Methods/ approach**: In an IRB approved investigation, 5 healthy adult males underwent an 8 minute MEG scan with an accompanying 3 lead ECG setup. After preprocessing the MEG time series, the data was fed through a convolutional neural network to identify and isolate the heart rate signal. This signal was extracted and subsequently quantified using the Kubios Premium software package. This methodology was tested against gold-standard ECG recordings to ensure accuracy for future heart rate artifact extraction and quantification. **Results**: The technical development scans indicate our method is reliably capturing R-R intervals of heart rate data. After thoroughly examining for aberrant beat detection, the ECG and MEG-extracted heart rate signal were significantly correlated using a simple linear regression to compare variances in the signal throughout the scans. ($P<.001$, RMSE>3ms)**Conclusion/ implications**: This provides a potential method for efficiently extracting HRV from MEG scans to provide information on autonomic biomarkers

Disclosures: W. Flood: None. **R. Godwin:** None.

Poster

668. Computational Tools for Experimental Studies

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Program #/Poster #: 668.12

Topic: I.07. Data Analysis and Statistics

Support: NIH-R01MH111438

Title: Mathematical denoising of 0.5Hz EPI-fMRI images with 100 micron isotropic resolution

Authors: *X. LIU, D. HIKE, Z. XIE, Y. JIANG, B. ZHANG, N. KOONJOO, M. ROSEN, X. YU;
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Abstract: There is a growing trend of awake mouse functional Magnetic Resonance Imaging (fMRI) to study the cross-scale brain function across a large pool of transgenic mouse lines. Achieving high-resolution mouse brain functional images is a challenge while typical mouse fMRI images have a 200x200 μm in-plane resolution with sub-millimeter thickness. Using a novel implantable MRI coil with an ultra-high field scanner, we obtained awake mouse Echo-Planar-Imaging (EPI) fMRI images with 100μm isotropic resolution. This method enables brain-wide single-vessel fMRI mapping (Yu X, et al. *Nat methods*. 2016; He Y, et al. *Neuron*. 2018; Chen X, et al. *Nat Commun*. 2019.) to decipher vasodynamic responses across different disease mouse models. The signal-to-noise ratio (SNR) of the raw fMRI images is limited given the high spatiotemporal resolution, so we used four different denoising methods (Ma X, et al. *Neuroimage*. 2020; Veraart J, et al. *MRM*. 2016; Aminghafari M, et al., *Comput Stat & Data An*.

2006; Donoho DL, *IEEE T inform theory*. 1995) to compare their efficiency to improve the SNR, and noise residues in time series. A horizontal 14T scanner was used to obtain 2D gradient echo EPI (TE=7ms, TR=1s with two segments, NR=200) fMRI images coupled with implanted ellipsoid-shaped surface coils (D = 9mm L-R, 11mm R-C) on awake mice. **Fig 1A&B** show comparisons of the raw coronal data and four images processed with different denoising methods (MPPCA, VST, 1-D wavelet, and Multivariate wavelet) and their removed noise. **Fig 1C** shows the region of interest (ROI) to calculate the SNR (**Fig 1D**) and tSNR (**Fig 1F**), and **Fig 1E** shows the time series of the raw and four denoised fMRI in the ROI. Although MPPCA shows a relatively lower SNR, its noise residual maps present a white-noise-like pattern, and its low tSNR indicates that more temporal information is retained. This work supports further exploration of machine learning-based denoising schemes that improve SNR while minimizing interference with the true signal of MRI images, and also the implementation of concurrent neural network-based denoise schemes for resting-state fMRI.

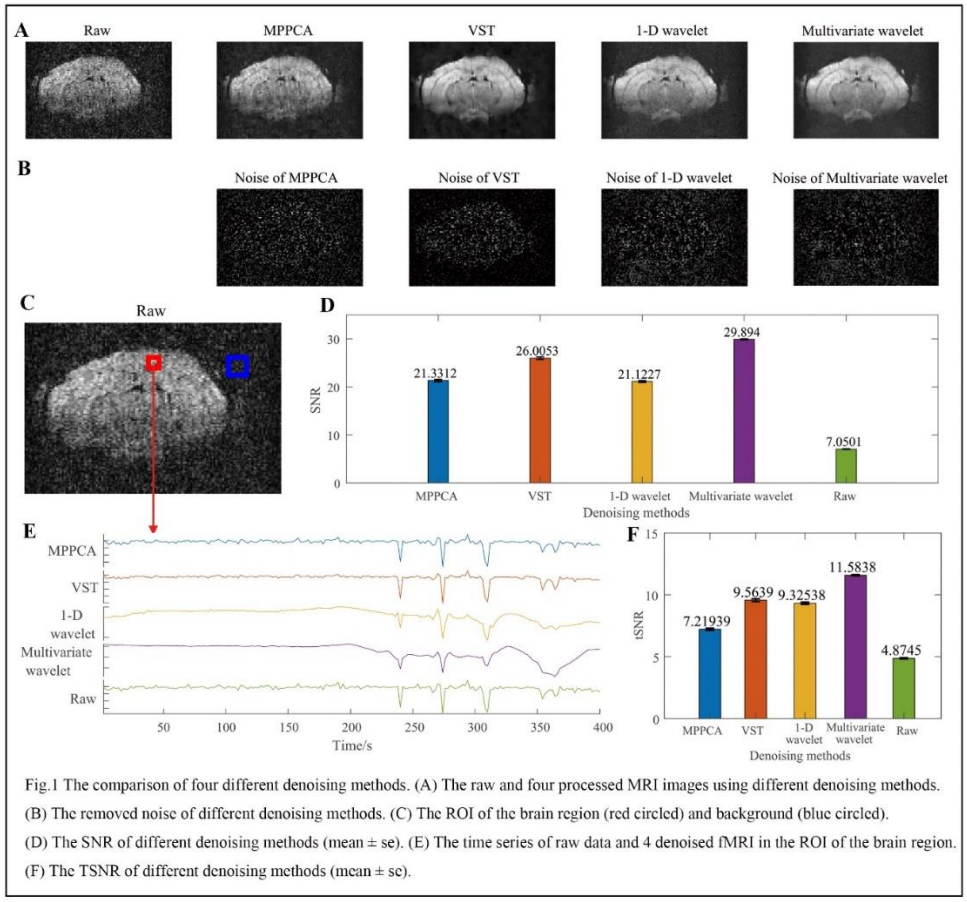

Fig.1 The comparison of four different denoising methods. (A) The raw and four processed MRI images using different denoising methods. (B) The removed noise of different denoising methods. (C) The ROI of the brain region (red circled) and background (blue circled). (D) The SNR of different denoising methods (mean ± se). (E) The time series of raw data and 4 denoised fMRI in the ROI of the brain region. (F) The TSNR of different denoising methods (mean ± se).

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Poster

668. Computational Tools for Experimental Studies

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Program #/Poster #: 668.13

Topic: I.06. Computation, Modeling, and Simulation

Support: MR/N013840/1

Title: AutoMacq: an automatic pipeline to analyse macaque structural MRI data

Authors: *N. KINDRED[1], D. ARDESCH[2], M. VAN DEN HEUVEL[2], F. BALEZEAU[1], Y. WANG[1], C. POIRIER[1];
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Abstract: Background: Non-human primate MRI is a rapidly growing area of research that promises to transform and scale translational and cross-species comparative neuroscience. Easy to implement pipelines that require little to no manual intervention are well established for human MRI data. However, this is not the case for MRI data from macaques, the main non-human primate model species, which comes with added complications and cannot simply be processed using the same pipelines as those developed for human data.
Study Objective: To develop an analysis pipeline that could accurately process both cross-sectional and longitudinal structural macaque MRI data, to produce both voxel-based and surface-based metrics, with minimal manual intervention.
Methods: The program SPM was utilised to incorporate voxel-based morphometry into the pipeline, and FreeSurfer was used to incorporate surface-based morphometry. N4 debiasing through ANTs, as well as the Bias Field Correction script used by the Human Connectome Pipeline, were included to reduce noise and improve accuracy. The pipeline was tested using data from different sites covering a wide array of scan parameters. Both cross-sectional (N=134) and longitudinal (N=17) datasets were utilised. Two different approaches to assess the reliability of the pipeline were carried out: 1) comparison of surface-based metrics between hemispheres (N=134); 2) comparison of whole brain metrics using scan-rescan data for a subset of subjects (N=12).
Results: Both longitudinal and cross-sectional datasets have been successfully processed through the pipeline without computational errors, with volume-based and surface-based outputs being obtained for all of the datasets. Outputs were obtained without manual correction of grey matter segmentation, and visual inspection of these outputs, as well as correlational analyses of within-subject hemispheric metrics (R=0.82-0.85) and scan-rescan whole-brain data (R=0.83-0.95), illustrate a high degree of reliability and accuracy.
Conclusions: AutoMacq provides a relatively easy to use pipeline for analysing macaque MRI data. The pipeline is novel in its incorporation of both voxel-based and surface-based morphometry, and involves minimal manual intervention which does not require any expertise in brain anatomy.

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